

**STUDY ON HISTOLOGICAL ALTERATIONS IN SELECTED
WILD FISH POPULATION INHABITING SEWAGE
DISPOSAL SITE IN THERESPURAM OFF THOOTHUKUDI,
SOUTHEAST COAST OF INDIA**

*Thesis submitted in part fulfillment of the requirements for the Degree of
Master of Fisheries Science in Fisheries Resource Management
to the Tamil Nadu Fisheries University, Nagapattinam*

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CERTIFICATE

This is to certify that the thesis entitled, **“Study on histological alterations in selected wild fish population inhabiting sewage disposal site in Therespuram off Thoothukudi, Southeast coast of India”** submitted in part fulfillment of the requirements for the degree of **Master of Fisheries Science in Fisheries Resource Management** to the Tamil Nadu Fisheries University, Nagapattinam is a record of bonafide research work carried out by **Ms. T. KALAIYARASI, I.D. No. MFT 15067 (FRM)** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that part of the thesis has been published in peer reviewed journal(s) and copy / copies appended.

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**DEDICATED TO MY
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T. KALAIYARASI

ABSTRACT

Title	:	Study on histological alterations in selected wild fish population inhabiting sewage disposal site in Therespuram off Thoothukudi, Southeast coast of India
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The present study aimed at assessing the effects of sewage pollution on finfish and shellfish species composition, physico-chemical properties of seawater and histology of various organs of fishes like *Arius maculatus* and *Terapon puta* collected from sewage disposal site in Therespuram off Thoothukudi coast. For studying species composition, finfishes and shellfishes were collected bimonthly from the sewage disposal site, Therespuram off Thoothukudi from September 2016 to June 2017 by gillnetting. A total of 400 specimens that included several finfish, shellfish and sea urchin species were collected during the study period. This included 30 species of finfishes, 8 species of shellfishes and one species of sea urchin. The finfish order, Siluriformes was observed to dominate with most number of specimens collected (65 %), followed by Perciformes (22.25 %). Among the species, *A. maculatus* was found to dominate with 31% of specimens collected, followed by *T. puta* (12.5 %). For the analysis of physico-chemical properties of seawater, seawater samples were collected from the study sites (five different spots

designated as T₁, T₂, T₃, T₄ and T₅ at sewage disposal site, Thersepuram, off Thoothukudi coast) and the reference site (Vembar) and were analysed and compared. The parameters analysed included temperature, salinity, pH, alkalinity, biological oxygen demand, chemical oxygen demand, total dissolved solids, total suspended solids, ammonia, nitrate, nitrite, phosphate and silicate. Statistical analysis (one way ANOVA) showed significant difference between any two study sites and also between anyone of the study site and reference site and also between different seasons at (P<0.05) level except temperature. However, all the parameters were found to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site and also between different seasons (P<0.05) and (P<0.01) level respectively. Further, spread rate map was prepared for various physico-chemical properties of seawater in GIS platform using Arc-GIS software, to know the spread rate of sewage pollution at the sewage disposal site.

Heavy metals such as copper, cadmium, lead and nickel were estimated in seawater samples collected from the exact point of sewage disposal at Thersepuram, off Thoothukudi coast (T₁) and reference site (Vembar) during summer season. The bioaccumulation factor (BAF) was calculated for each heavy metal for both the fish species. The BAF was higher for *A. maculatus* than *Terapon puta* collected from study site. With regard to heavy metal, the highest BAF was observed for Cadmium in gill tissues of *T. puta*.

Histological sections of gill, liver, kidney, brain and muscle tissues of *A. maculatus* and *T. puta* collected from sewage polluted study site and relatively

unpolluted reference site were observed under light microscope. histological alterations occurred in the above-mentioned organs of these two species collected from sewage disposal site were compared with the histology of respective organs of the same species collected from relatively unpolluted reference site (Vembar) and histopathological changes were marked. These histological alterations can serve as biomarker for monitoring the quality of seawater at the sewage disposal site.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	-	Analysis of Variance
BC	-	Blood Congestion
BEC	-	Breakdown of Epithelial Cells
BEL	-	Breakdown of Epithelial Layer
BiC	-	Bile Canaliculi
BmC	-	Bowman's Capsule
BN	-	Binucleated Nucleus
BS	-	Blood Sinusoid
BV	-	Blood Vessel
Cd	-	Cadmium
CN	-	Cellular Necrosis
DG	-	Degenerated Glomerulus
DN	-	Degenerated Nucleus
DSL	-	Degeneration of Secondary Lamellae
DT	-	Degenerated Tubules
EpL	-	Epithelial Lifting
Er	-	Erythrocyte
ERH	-	Epithelium Rupture with Hemorrhage
ESL	-	Erosion of Secondary Lamellae
G	-	Glomerulus
H	-	Hyperplasia
H & E	-	Haematoxylin and Eosin
HeT	-	Hepatic Tissue
HT	-	Hematopoietic Tissue
IKC	-	Increased Kupffer Cells
IPGs	-	Increased Periglomerular Space
IPN	-	Increased Pycnotic Nucleus
IPTs	-	Increased Peritubular Space
KC	-	Kupffer Cells
LC	-	Loss of Cytoplasm

LF	-	Lamellar Fusion
M	-	Melanomacrophages
ME	-	Mucous Exudate
Nu	-	Nucleus
PGL	-	Primary Gill Lamellae
PLP	-	Phospholipid
PN	-	Pycnotic Nucleus
RBC	-	Rupture of Bowman's capsule
RH	-	Rupture of Hepatocytes
RN	-	Ruptured Nucleus
RT	-	Renal Tubules
SBM	-	Separation of Boundary Membrane
SBV	-	Shrinkage of Blood Vessels
SG	-	Shrunk Glomerulus
SGL	-	Secondary Gill Lamellae
SLD	-	Secondary Lamellar Damage
USEPA	-	U.S. Environmental Protection Agency
V	-	Vacuolation

I. INTRODUCTION

Background

Aquatic pollution is one of the current global environmental issues. Water resources have been the most exploited natural systems since man strode the earth. With the rapid development of industrialization, unplanned urbanization and an increase in human population, the pollution of water bodies has become a universal phenomenon in the present day world. The discharge of industrial, agricultural and domestic waste water into the environment results in the pollution of these aquatic systems.

The United Nations Convention on the Law of the Sea defined 'Pollution' as the introduction by man, directly or indirectly, of substances or energy into the marine environment, including estuaries, which results or is likely to result in such deleterious effects as harm to living resources and marine life, hazards to human health, hindrance to marine activities, including fishing and other legitimate uses of the sea, impairment of quality for use of the sea water and reduction of amenities. These anthropogenic activities lead to depletion of oxygen in water, elevated BOD load, and changes in transparency, pH, phosphate, nitrate, COD and mineral concentration causing eutrophication, thus degrading water quality. This unabated exploitation of ecosystem is exerting tremendous strain on aquatic communities including plankton, fishes and other invertebrates (Zutshi and Prasad, 2008). Thus, water bodies are frequently stores for a large variety of xenobiotics which cause the biochemical and histopathological alterations in fish (Bernet et al., 1999 and Reddy, 2012).

Coastal pollution

Coastal environment plays a major role in the wealth of the nations by asset of the resources, productive habitats and rich biodiversity. However, fish are often exposed to highly contaminated water, especially in areas where the dilution rate of waste water is low. In fish, water pollution can lead to different changes ranging from biochemical alterations in single cells up to changes in whole populations (Bernet et al., 1999). According to the World Resource Centre report (2016), coastal habitats alone account for approximately 1/3 of all marine biological productivity. Around 20% of human population inhabit in the coastal zone around the world. In case of India, around 30% of people are living in the coastal zone. 40% of the world's oceans have been heavily affected by human activities, including fishing, coastal development and pollution from shipping. Over 80% of all marine pollution originates from land based source which are primarily industrial, agricultural and urban.

Sewage is a complex mixture of chemicals. These include high concentrations of ammonium, nitrate, phosphorus, high conductivity (due to high dissolved solids), high alkalinity, with pH typically ranging between 7 and 8. This high concentration of nutrients causes eutrophication in coastal waters.

The coastal pollution has seriously affected the exploitable living resources, recreational and commercial uses of coastal areas and the overall integrity of the marine and coastal ecosystems. Hence, the protection of the coastal and marine regions from continuing pollution becomes the most essential in coastal resources management (Hamre et al., 2009). Effective planning for controlling and combating coastal pollution requires knowledge about the magnitude of the pollution, the entry,

transport and state of pollutants in the marine environment and their effects on marine ecosystems.

Pollution from harbour generated wastes can be categorized as those that cause: visible pollution of land and water by oil spills and sewage; invisible pollution of harbour water by hazardous wastes; and degradation of the harbour environment by discarded litter and fish offal (Sciortino and Ravikumar, 1999). Among them, the most predominant one is the oil pollution. While supplying diesel fuel to boats, leaking-hoses and careless handling result in spills that invariably find their way into the harbour water. Similarly, dumping of used engine oil and pumping of oily bilge water into harbour water is very often the cause for oil (hydrocarbons) pollution in fishing harbours (Ravikumar, 1993).

Biomarkers for pollution monitoring

Chemical monitoring of water helps to measure the degree of contamination. But for the assessment and evaluation of pollution effects on various organisms including fish can be studied by using biomarker response. Biomarkers are measures of sub-organismal responses in organisms or exposed biological systems which can demonstrate exposure to, or the effects of environmental contaminants (Peakall, 1992). Biological and ecological responses to contaminant stressors may range from changes at the molecular level, where genetic integrity and subcellular processes are evaluated, to population and community levels where dynamics and structure of entire food webs can be affected (Adams, 1990; Shugart *et al.*, 1992). Indicators of stress at several levels of biological organization have been used to evaluate effects of contaminants at the organism level. These approaches vary from

measures of genetic integrity to growth and reproductive competence of the individual. There are both advantages and limitations to every approach or measurement which is used to evaluate the effects of stress on organisms (Adams, 1990).

Over the past two decades, the science of biomarkers has advanced considerably (SETAC, 1992), and there is a worldwide trend to supplement chemical and physical parameters with biomarkers in marine pollution monitoring (ANZECC, 1992). A variety of molecular, biochemical, physiological, histo-cytopathological, organismal, population and community responses may be used to identify exposure to certain chemicals, provide information on spatial and temporal changes in the concentration of contaminants, and indicate environmental quality or occurrence of adverse ecological consequences. In general terms, responses at lower biological organization levels (e.g., molecular and biochemical responses) are more specific, sensitive, reproducible and easier to determine, but more difficult to relate to ecological changes and generally lack realism. On the other hand, responses at higher biological organization levels (e.g. population and community responses) are directly indicative of ecosystem health and hence, much more relevant to environmental management. However, they are more difficult to determine, less specific and only manifest at a late stage when environmental damages have already occurred (Connell *et al.*, 1999).

Histology as a biomarker for pollution monitoring

Most biomarkers are narrow in their expression whereas histopathology is broad in its evaluation (Moore and Myers, 1994). Histopathological changes in

animal tissues are reliable and direct indicators to environment stressors (Malik et al., 2012). It is also the easiest method of assessing both short and long term toxic effects (Hinton, 1995). Histopathological evaluation is a sensitive tool in toxicant impact assessment to indicate the effect of toxicants on fish health and also allows for early warning signs of disease and injury in cells, tissues, or organs. Such structural changes in fish as biomarkers in various tissues in different species have also been studied by many researchers. Therefore, Histopathology has become a standard biomarker when defining toxicological effects, but it is invasive, time consuming and expensive. Yet, histopathological evaluation remains an important part of the assessment of the adverse effects of xenobiotics on the whole organism (Reddy and Rawat, 2013).

Histopathological characteristics of specific organs express condition and represent time-integrated endogenous and exogenous impacts on the organism stemming from alterations at lower levels of biological organization (Chavin, 1973; Stebbing, 1985). Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters and, as an integrative parameter, provide a better evaluation of organism health than a single biochemical parameter (Segner and Braunbeck, 1988). Effects of marine pollution on fish health have been reviewed by Austin (1999), whereas the application of histo-cytopathological biomarker in marine pollution monitoring has been reviewed by Au (2004).

Additionally, histological biomarkers provide powerful tools to detect and characterise the biological end points of toxicant and carcinogen exposure (Hinton

and Lauren, 1990; Wester and Canton, 1991; Hinton, 1992; Moore and Simpson, 1992) and oil exposure (Simanato *et al.*, 2008; Agamy, 2012; Gusmao *et al.*, 2012; Omar *et al.*, 2012; Khabaksh *et al.*, 2014 and Kazempoor *et al.*, 2015). As such, the utility of histological lesions as sensitive and reliable indicators of the health of wild fish populations has been demonstrated in several Indian studies. Several laboratory and mesocosm studies have also demonstrated causal links between exposure to xenobiotics and the development of toxicopathic hepatic lesions.

The use of histopathological markers has already been tested and proposed as an efficient and sensitive method to monitor fish health and environmental pollution in natural water bodies (Au, 2004; Ayas *et al.*, 2007; Camargo and Martinez 2007; Costa *et al.*, 2009, 2011; Leonardi *et al.*, 2009). Such biomarkers might indicate acute or chronic exposure to contaminants and facilitate detection of fish physiological responses, thereby establishing a more realistic diagnosis for evaluating environmental health (Silva *et al.*, 2009).

Objectives

Thoothukudi which is present in Gulf of Mannar has a greatest interest due to an industrial belt having many major industries, involved in the production of chemicals, petrochemicals and plastics. The human activities around the Thoothukudi have altered the coastal ecosystem greatly. The municipality generates sewage of 17.5 MLD capacity (Asha et al, 2009). This sewage is discharged into the sea without any treatment (Tamil Nadu Fisheries Statistics, 2004). Hence, the present research work aimed at studying the histological alterations in *Arius*

maculatus and *Terapon puta* inhabiting the sewage disposal site in Therespuram off Thoothukudi, Southeast coast of India with the following objectives.

1. To prepare a checklist of various fish species inhabiting sewage disposal site (SDS) in Therespuram off Thoothukudi
2. To analyse the temporal variations in the selected physico-chemical properties of sea water in SDS in Therespuram off Thoothukudi and in a relatively unpolluted reference site (UPRS)
3. To compare the histological alterations that are expected to take place in the various organs of selected fish population inhabiting SDS in Therespuram off Thoothukudi with the histology of respective organs of fish population inhabiting the UPRS

II. REVIEW OF LITERATURE

Literature on impacts of various kinds of pollutants on histology of different organs of fishes from wild aquatic environment and from both *in vitro* and *in vivo* studies and potential mechanism / modes of action of various pollutants have been critically and exhaustively reviewed by several investigators (Lagadic et al., 2000; Winkaler et al., 2001; Adams, 2002; Oga, 2003). The present review summarizes the impact of pollution particularly sewage pollution on the histology of gill, liver, kidney, brain and muscle tissues of fishes.

2.1. Pollution of coastal and marine ecosystem

Coastal and marine ecosystems are one of the most productive ecosystems in the world, provide many services to human society and are of great economic value (UNEP, 2006). Seventy five percentage of commercially harvested fish and shellfish depends on estuary and nearby coastal water for some part of their life cycle. Water quality of the coastal areas, estuaries and major river around the world is getting rapidly degraded due to massive discharge of industrial water of diverse origin, domestic sewage, mine drainage, oil spills, extensive use of agrochemicals (Zutshi and Prasad, 2008).

Coastal and marine ecosystems in the present world are being severely affected by rapid growth of human population and intensive urbanization in coastal regions. Hence, pollution in marine environments had become one of the serious issues and it has been expanded all over the marine ecosystem from deep sea to coastal waters. Most of the industries in the world are settled in coastal belts of the

countries, especially, around major commercial harbours and airports due to ease of trade, which contribute to severe pollution (Niroshana, 2013).

The economic importance of coastal areas has increased further with the growth of human settlements and the development of commercial harbours, fishery harbours, landing facilities, river training and outfall schemes, transport, communications, recreational facilities and tourism (CZMP, 1990). These accelerating urbanization and industrialization in coastal areas lead to substantial increases in the aggregate amounts of wastes produced and thus waste management and coastal water pollution control are of increasing concern (CZMP, 2007). The wide diversity of human activities introduce pollutants into the environment, as well as their magnitudes make the assessment of environmental impact a subject of utmost interest (Marcovecchio, 2004). The presence of metals in aquatic ecosystems originates from the natural interactions between the water, sediments and atmosphere (Kalay and Canil, 2000; Sankar et al., 2006). Aquatic organisms are widely used to monitor environmental health due to anthropogenic impacts (Hellawell, 1986; Evans et al., 1993; Rashed, 2001).

Agricultural, industrial and domestic effluents generally contain a wide variety of organic and inorganic pollutants, such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids (Pandey et al., 2003) and are, invariably, discharged into small rivers and streams, without proper treatment. Such contaminants change water quality and may cause many problems to fish. Fishes have been proposed as sentinel species for the biomonitoring of land-based pollution because they may accumulate hydrophobic organic compounds in their tissues, directly from water,

sediments and/or through their diets. Heavy metals accumulate in tissues and may pose a health risk to those who frequently consume fish. In the organism, xenobiotic compounds undergo a series of biotransformation reactions catalyzed by different enzymatic systems; their activation may provide additional evidence for pollution exposure (Mormede and Davies, 2001). Intensive industrial and agricultural activities have inevitably increased the levels of heavy metals in natural waters (Jordao et al., 2002). For these reasons, it is important to determine the concentrations of heavy metals in commercial fish in order to evaluate the possible risk of fish consumption for human health (Cid et al., 2001).

2.2. Coastal sewage pollution

The problems associated with sewage disposal have become a major problem of the urban world due to increase in human population and urbanization. The commonality of sewage related problems throughout coastal areas of the world is significant since these areas are inhabited by over 60% of the human population. Consequently, domestic waste-water discharges are considered one of the most significant threats of the coastal environments worldwide (GPA, 2001). Environmental effects associated with domestic waste-water discharges are generally local with transboundary implications in some areas. Coastal waters are facing a variety of pressure affecting both the ecosystem and human health through sewage waste-water discharge and disposal practices that may lead to introduction of high nutrient loads, hazardous chemicals and pathogens causing diseases. The adverse public health, environmental, socio-economic, food quality and security, and aesthetic impacts from sewage contamination in coastal areas were well

documented (Luger and Brown, 1999; Tyrrel, 1999; Danulat et al., 2002; WHO, 2003). Pollution of the coastal water usually interferes with various water uses. Every pathogen present in seawater may be trapped and concentrated in the tissues of the cultured bivalves and so represents a potential health hazard (Owili, 2003).

2.3. Effects of pollution on physico-chemical properties of water

Water quality deterioration is one of the most important water resource issues of the 21st century. Therefore the quality status of coastal surface water is very important and would always be under public scrutiny because of health risk associated with sewage contamination. The potential deleterious effects of pollutants from sewage effluents on the receiving water quality of the coastal environment are manifold and depend on volume of the discharge, the chemical composition and concentrations in the effluent. It also depends on type of the discharge for example whether it is amount of suspended solids or organic matter or hazardous pollutants like heavy metals and organochlorines, and the characteristics of the receiving waters (NAP, 1984; Nemerow and Dasgupta, 1991; Canter, 1996).

Large quantities of nutrients released into the coastal water through the sewage waste-water may result in nutrient enrichment stimulating algal growth that in turn affects the photic zone depth, cause dissolved oxygen depletion, bioaccumulation of organic and inorganic compounds, and alteration of trophic interactions among both aquatic flora and fauna (Danulat et al., 2002; Russo, 2002). Elevated nutrient levels may also result in excessive growth of algal blooms, some of which may result in production of algal toxins. The algal toxins are risks for water and seafood quality and safety (Owili, 2003).

2.4. Application of GIS in aquatic pollution monitoring

Water quality of ocean and inland waters using remote sensing satellite is not recent and it has been assessed using Landsat Multispectral Scanner (MSS) since early 70s (Ritchie et al., 1990; Thiemann and Kaufmann, 2000). Today, with the advent of advanced technologies, better satellites available with high resolutions are used for monitoring water quality studies (Ritchie et al., 2003). Chandar and Kumar (2012) analysed the marine pollution in Tuticorin coastal areas using remote sensing and GIS. In their study, they analysed the spread rate and hotspot with the aid of various physical, chemical, biological parameters of water. Vijay et al. (2015) studied that extent of sewage pollution in coastal environment of Mumbai for better planning and management by using object based image analysis.

2.5. Bioaccumulation of heavy metal in marine organisms

Raghavan (2006) studied the bioaccumulation of heavy metals in edible oyster *Crassostrea madrasensis* collected from Thoothukudi coast off Gulf of Mannar. Reddy et al. (2007) undertook a study on bioaccumulation of heavy metals in commercial fishes and crabs of Gulf of Bombay. Asha et al. (2010) investigated the heavy metal concentration in seawater, sediment and bivalves along the Thoothukudi coast. Obadiah et al. (2010) investigated the bioaccumulation of heavy metal like As, Pb, Cd, Cu and Cr in muscle tissue of fishes collected from Tuticorin coastal area, Gulf of Mannar, India. Khaled (2014) determined the heavy metal concentration in muscle tissue of fishes such as *Siganus rivulatus* and *Scatophagus argus* collected from El-max Bay and eastern harbour in Egypt.

2.6. Effects of sewage pollution on marine organisms

Sewage pollution promotes hypoxia, or oxygen depletion, in aquatic ecosystems in two ways. Firstly, sewage itself contains large amounts of organic matter which is directly available to bacteria in the water. Secondly, it promotes growth of plants and algae that become a source of organic matter, when they die. When bacteria consume organic matter they also consume dissolved oxygen from the water. Hypoxia can kill animals or cause physiological stress that stunts growth and reproduction (Mearns et al., 2013).

A common short-term response by fish to a sewage outfall is an initial increase in abundance around the point of discharge. There is a short-term increase in nutrients and, hence, prey items for the fish and, on occasions an increase in habitat complexity, which may cause an initial population rise in fish species. Yet, as nutrient levels increase so does the chance of algal bloom development, toxin production and a corresponding decrease in dissolved oxygen. Long-term effects include phytoplankton biomass increases and large scale decreases in species diversity with benthic and fish communities (Bonsdorff et al., 1997).

Azzurro et al. (2010) observed a huge difference between fish assemblage and species composition in sewage polluted coastal sites and an increase in small opportunistic species and decrease in other commercially important species. In addition, they recorded a significant change in fish size distribution for several species in sewage polluted coastal sites. A long-term monitoring study in Kenting National Park (Taiwan) revealed declining water quality and adverse effects on coral communities resulting from increased sewage discharges (Liu et al., 2012).

Pastorok and Bilyard (1985) noted that sewage pollution can severely affect the coral reef ecosystem and pollutants in the sewage water increase abundance of benthic algae and filter feeding invertebrates in the coral reef ecosystem. Pitanga et al. (2012) reported that seagrass meadows (*Halodule wrightii*) in north eastern Brazil were affected by several anthropogenic factors, including the discharge of untreated sewage. Benthic macro invertebrate assemblages near two fish-processing plant discharges in New Brunswick (Canada) showed reduced taxa richness and density when compared with areas away from the outfalls (Lalonde and Ernst, 2012).

A study of mesozooplankton in a eutrophic estuary receiving sewage discharges in Argentina indicated an enhanced abundance of detritivorous crab larvae (*Neohelice granulata*) when compared to a reference estuary (Dutto et al., 2012). Cross and Hose (1988) reported that there was a decline in the catches of commercial and sport fishes catch in the Los Angeles due to domestic and industrial water discharge in the coastal areas. They also reported impaired reproduction in white croaker (Sciaenidae: *Genyonemus lineatus*) because of domestic and industrial water discharge in the coastal areas.

2.7. Biological markers of environmental contamination

Biomarkers are defined as biochemical or cellular response that can be measured and that indicates that the organism has been exposed to a chemical or anthropogenic stress. Biomarkers are measured in organisms collected from (or confined to) sites of suspected contamination and the responses are compared to those from the same species collected from pristine reference sites (Moore et al., 2004).

The presence of xenobiotics in the marine environment exerts well-known biological effects on marine organisms. Occasionally, when properly evaluated in selected sentinel species, these effects may be considered as biomarkers, a sort of early warning signaling useful in assessing the quality of marine habitats (Bayne et al., 1985; Cajaraville et al., 2000; Lam and Gray, 2003; Moore et al., 2004). Biomarkers have been proposed as sensitive tools for the early detection of environmental exposure to pollutants and their adverse effects on aquatic organisms (Oost et al., 2003; Torre et al., 2005). They also serve as links between the environmental contamination (cause) and its effects, providing unique information on the ecosystem health (Maria et al., 2009). In the past 25 years, numerous biomarkers have been developed with the objective to apply them for environmental biomonitoring (McCarthy and Shugart, 1990; Peakall, 1994; Shugart and Theodorakis, 1998).

2.8. Histology as a biomarker

The response to chemical stress can be used as biomarkers or sentinels of environmental conditions. Biomarkers are early responses or measurable biological event due to exposure to pollutants after acute or chronic exposure and the morphological findings has been largely considered in biomonitoring studies (Adams, 2002; Lagadic et al., 2000; Oga.,2003; Winkaler et al., 2001; Ribeiro et al., 2005; Miranda et al., 2008). According to Huggett et al. (1992), the most common usage of the term biomarker has been for biochemical, physiological or histological indicators of either exposure to or the effects of xenobiotic chemicals at the sub-organism or organism level. The advantage of histopathology as a biomarker lies in

its intermediate location with regard to the level of biological organization (Adams et al., 1989). Histological changes appear as a medium term response to sub-lethal stressors, and histology provides a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs (McCain and Varanasi, 1993).

Histopathological events are considered fast and efficient for detection of acute and chronic adverse effects in fish; and express the health condition of exposed individuals (Myers and Fournier 2002; Oliveira Ribeiro et al., 2005; Miranda et al., 2008). Field investigations are important to interpret assessment risks and understand biological effects of chemicals under natural conditions, representing also a real health state (Mallatt, 1985; Lagadic et al., 2000; Winkaler et al., 2001; Alberto et al., 2003).

Toxicant effects can be studied at different levels of biological organization. Many authors considered that the different changes in many biochemical and morphological parameters of fish may be used as successful biomarkers for toxic effects of xenobiotics (Bernet et al., 1999; Monteiro et al., 2005; Cazenave et al., 2009; Marchand et al., 2009). Initial effects of toxicant pollution may be evident only at cellular or tissue levels before significant changes are identified in fish behaviour or external appearance. Hence, the histological alterations have been examined for decades in fish tissues and organs in order to assess the effects of pollutants (Johnson et al., 1993; Stentiford et al., 2003; Au, 2004). As an indicator of exposure to chemicals, histology represents a useful tool to assess the degree of pollution (Perry and Laurent, 1993). The histopathological changes have been widely used as biomarkers for the evaluation of the health of fish, exposed to contaminants in

laboratory conditions. One of the important advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs (Wester and Canton, 1991).

Histopathological changes have been integrated with the impact of various stressors (microbial pathogens, toxic compounds, nutritional and adverse environmental conditions (Yancheva et al., 2013). Histopathological characteristics of specific organs express condition and represent time-integrated endogenous and exogenous impacts on the organism stemming from alterations at lower levels of biological organization. Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters and, as an integrative parameter, provide a better evaluation of organism health than a single biochemical parameter (Chavin, 1973; Stebbing, 1985).

2.9. Histopathology of fishes exposed to pollution

Fishes are considered as early warning for the degradation of environmental quality but also specific measures of the existence of toxic carcinogenic and mutagenic compounds in the biological materials. Liver and gills as main organs for metabolism and respiration are target organs for contaminants accumulation as reported by many authors concerning structural damage to organs and tissue related to exposure of fish to various pollutants. Untreated sewage effluents consist of complex mixtures of toxicants that increase significantly the impact on aquatic ecosystems, decreasing the species diversity in different regions of the world (Schmitt et al., 1999; Amisah and Cowx, 2000). The tolerance of wild organisms to

toxicants in domestic effluents may vary among species and their integrative effects may lead to reproductive failure or reduction of fish species number (Bonga, 1997; Minier et al., 2006). Many researchers have reported the degenerative changes in selected tissues of the fishes in response to different kinds of toxicants (Lagadic et al., 2000; Winkaler et al., 2001; Adams, 2002; Oga, 2003; Ribeiro et al., 2005; Miranda et al., 2008).

2.9.1. Histopathology of fish gill

Fish gill is a multifunctional organ responsible for respiration, osmoregulation, acid–base balance and nitrogenous waste excretion. This organ is sensitive to chemicals in water, since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water. It is particularly sensitive to chemical and physical changes of the aquatic environment, thereby being the target organ in fish for pollutants carried by water (Mallatt, 1985; Mazon et al., 1999; Cerqueira and Fernandes, 2002). Concentrations of several pollutants seriously damage the gills of teleostean fish (Wenderlaar, 1997). Gill histopathology appears to be a promising biomarker for environmental contamination. Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism (Hanan, 2013).

Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenol and heavy metal. Gill histopathological changes are, in general, responsive but non-specific to pollutant exposure (Nowak et al., 1992). Mallatt (1985) reviewed the most common gill lesions found under several stressful conditions, and concluded that the most commonly reported changes (epithelial lifting, necrosis, hyperplasia, hypertrophy and haemorrhage) were results of lethal conditions.

Pugazhvendan et al. (2009) observed histological changes in gill tissue of *Ophiocephalus punctatus* exposed to malathion pesticide. The changes observed were curled lamellae, edema and vacuolation. Chamarthi et al. (2014) studied the histological alterations in gill tissue of *Cyprinus carpio* exposed to quinalphos. Coulibaly (2012) investigated the histological changes in gills of black finned tilapia from heavy metal contaminated Bay Bietri, Brazil. Marchand (2012) reported that histopathological changes of fish gill from polluted freshwater system that revealed aneurysms, epithelial lifting, rupture of pillar cells, and hyperplasia of epithelial and mucous cells. Gusma et al. (2012) reported that the larvae of Marine Pejerrey (*Odontesthes argentinensis*) exposed to water soluble fraction of petroleum and resulted in histological alterations like aneurysm, lifting of the respiratory epithelium, hyperplasia in the gill tissue of the larvae.

Liebal et al. (2013) noted that the fishes that were impacted by domestic sewage effluents showed histological alterations like gill aneurysm, disruption of pillar cells, and hyperplasia on epithelial tissue, primary lamellar fusion.

Consequently, this will decrease the surface area for gas exchange. Patnaik (2010) observed histological alterations in gill tissue of common carp exposed to heavy metals like Pb and Cd. Ibrahim (2013) reported the effect of water quality changes on histology of gills of *Oreochromis niloticus*. Mohammad et al. (2016) reported that *Siganus rivulatus* (Rabbit fish) collected from polluted site exhibited histological alterations in gill like, epithelial lifting, hyperplasia and hypertrophy of the respiratory epithelium, lamellar fusion and aneurysms.

2.9.2. Histopathology of fish liver

Teleost liver is the primary organ for biotransformation of organic xenobiotics, and also for the excretion of harmful trace metals, food digestion and storage, and metabolism of sex hormones (Health, 1995; Hinton et al., 2001). Histo-cytopathological changes occur in livers of fish exposed to a wide range of organic compounds and heavy metals. Livers of fish are sensitive to environmental contaminants because many contaminants tend to accumulate in the liver, making this organ exposed to a much higher levels (several orders of magnitude) than in the environment, or in other organs (Au, 2004).

The US National Marine Fisheries Services (USNMFS) conducted large scale surveys to determine the relationship between toxicopathic lesions in livers and exposure to chemical contaminants. The result revealed a good correlation between the concentrations of various persistent chlorinated hydrocarbons and metals, and liver lesions (Malins et al., 1988; Myers et al., 1992). Patnaik (2010) observed histological alterations of liver tissue of common carp exposed to heavy metals like Pb and Cd. The changes observed in liver tissue were hypertrophy, vacuolation, pyknotic nucleus and severe necrosis.

Pugazhvendan et al. (2009) observed histological changes in liver tissue of *Ophiocephalus punctatus* exposed to malathion pesticide. Chamarthi et al. (2014) studied the histological alterations in liver tissue of *Cyprinus carpio* exposed to quinalphos. The changes observed were necrosis, pyknotic nucleus, and central vein congestion. Marchand (2012) reported that histopathological changes occurred in liver of fishes collected from polluted freshwater system. Gusma et al. (2012) reported that the larvae of Marine Pejerrey (*Odontesthes argentinensis*) exposed to water soluble fraction of petroleum resulted in histological alterations like hypertrophy, karyopyknosis, enlarged sinusoids in the liver tissue of the larvae. Coulibaly (2012) noted histological changes in liver of black finned tilapia from heavy metals contaminated Bay Bietri, Brazil like dilation in hepatic cells, focal areas of necrosis and hepatic cells degeneration. And also, vacuolar degeneration with infiltration of lipid, blood vessels degeneration

Liebal et al. (2013) investigated the histopathology of fishes collected from sewage polluted lakes and observed histological alterations like leukocyte infiltration and necrosis in the liver. Mohammad et al. (2016) reported that *Siganus rivulatus* (Rabbit fish) collected from polluted site of Red sea showed the histological alterations in liver, like hepatocyte with hypertrophy, cytoplasmic and nuclear hypertrophy, melanomacrophage aggregates, bile stagnation and focal area necrosis. The presences of Macrophage aggregates in liver tissue of fishes exposed to contaminants have been suggested as potentially sensitive histological biomarkers and or immunological biomarker of contaminant exposure (Schmitt and Desthloft, 2000).

2.9.3. Histopathology of fish kidney

Kidneys of marine fish play an important role in maintaining osmotic homeostasis. Moreover, renal tissues receive large volumes of blood flow and serve as a major route of excretion for metabolites of various xenobiotics. Since renal tissues are potentially continuously exposed to toxic chemicals, the risk of effects is high. Non-specific kidney histopathological lesions (e.g. degenerative changes in tubular epithelium, dilation of tubular lumina, proteinaceous or cellular casts within tubular lumina, tubular necrosis and/or epithelial desquamation, and necrosis of interstitial hematopoietic tissues) were observed following exposure of fish to organochlorines, petroleum compounds, organophosphate, herbicides and heavy metals (Meyers and Hendricks, 1985; Global Tox, 1997).

Coulibaly (2012) observed histological changes in kidney of black finned tilapia from heavy metals contaminated Bay Bietri, Brazil. The changes observed were vacuolization, dilation in renal cells, severe dilation with renal cells degeneration, hemorrhage, parasitic cyst, vacuolar degeneration in the epithelium of renal tubules, parasites, and dilation in renal blood vessels. Ibrahim (2013) also reported the similar effect of water quality changes on histology of kidney of *Oreochromis niloticus*.

Gusma et al. (2012) reported that the larvae of Marine Pejerrey (*Odontesthes argentinensis*) exposed to water soluble fraction of petroleum resulted in histological alterations like enlargement of glomerulus, intercellular spaces and abnormal nucleus of epithelial cells, and hypertrophy.

2.9.4. Histopathology of fish brain

Loganathan et al. (2006) observed histological changes in brain of Rohu exposed to heavy metal, Zn. The changes observed were swelling of pyramidal cells with binucleated nucleus, enlarged pyramidal cells, vacuolation and necrosis. Pugazhvendan et al. (2009) observed histological changes in brain tissue of *Ophiocephalus punctatus* exposed to malathion pesticide. The changes observed were severe necrosis.

Patnaik (2010) observed histological alterations of brain tissue of common carp exposed to heavy metals like Pb and Cd. The changes observed in brain were neuronal cell degeneration, dystrophic changes, swelling of pyramidal cells, loss of nasal substratum and vacuolation. Chamarthi et al. (2014) studied the histological alterations in brain tissue of *Cyprinus carpio* exposed to quinalphos. The changes observed were neural cell intracellular edema and cytoplasmic vacuolation.

2.9.5. Histopathology of fish muscle

Ibrahim et al. (2013) studied bioaccumulation of heavy metals and their histopathological impact on muscles of *Clarias gariepinus* from El-Rahawy drain, Egypt. The result showed hyaline degeneration, edema, and splitting muscle bundles. Afifi et al. (2014) studied the histopathology of *Siganus canaliculatus* and *Epinephalus morio* caught from oil polluted Jeddah coast and the study revealed the histological alterations in muscle tissue like edema, hyalinization and necrosis. Rajeshkumar et al. (2015) assessed the heavy metal pollution in east Barbic-Correntyne, Guyana by using histology of *Mugill cephalus* muscle tissue. The result showed alterations like loss and swelling of muscle fibre layer, breakdown of muscle bundles, splitting of muscle fibres. A study was conducted by vasanthi et al. (2013) to assess the heavy metal pollution in Ennore estuary by using histology of muscle tissue of *Mugill cephalus*. The study revealed histological alterations like necrosis in the muscle bundles, connective tissue damage, splitting of muscle fibres (SMF) and edema between muscle bundles.

III. Materials and Methods

3.1. Study area profile

The coastal length of Thoothukudi District which is located along the southeast coast of India is 163.5 km, stretching from Vembar in the north to south of Manappad between 8° 9' 00" to 9° 7' 30" N Latitude and 78° 2' 30" to 78° 25' 00" E longitude (Plate 3.1). Thoothukudi, headquarters of the district is a port town with a population of around 0.4 million. As it is a port town, several chemical industries are in operation, discharging very large amount of effluents in to the sea. In addition, the town generates an estimated 17.5 MLD of sewage (Asha et al., 2009). There are no treatment facilities for the sewage. The entire sewage generated in the city is discharged directly in to the sea through a canal at Therespuram Lat 08° 48' 48 N and Long 78° 09' 48 E, which is one of the major fish landing centres situated in Thoothukudi district.

3.2. Reference site profile

Vembar is a coastal village, lying at Lat 09° 04' 35 N Long 78° 21' 54 E, in the northern part of Thoothukudi district (Plate3.1.). The fishermen of the village follow traditional fishing method and employ simple fishing gears which include trap, shore seine, gill net etc. As there is no major industrial activity taking place in and around Vembar, the costal water in this region is relatively unpolluted. Hence, Vembar has been selected as a relatively unpolluted reference site (UPRS) to compare the histology of various organs of *Terapon puta* (Cuvier, 1829) Small-scaled Banded Grunter and *Arius maculatus* (Thunberg, 1792) spotted catfish inhabiting this

unpolluted reference site with those from Therespuram, a polluted site for proving that histological alterations take place in the fishes inhabiting polluted region.

3.3. Collection of fish specimens

Fish sampling was conducted at sewage disposal site Therespuram off Thoothukudi and reference site (Vembar) during Sep' 2016 – June' 2017 bimonthly with the help of local fishermen using gill net. The fishes were collected and brought to the laboratory. The fish specimens were cleaned, photographed and finally preserved in 10% formalin. The standard literatures were referred for identification of fish species (Day, 1878, 1994; Smith, 1950; Munro, 1955; FAO species identification sheets). Fish base was followed to identify the fish species and their International Union for Conservation of Nature (IUCN) status.

3.4. Preparation of checklist

The fish species recorded from the sewage disposal site, Therespuram during sampling were classified order-wise and family-wise. Then, frequency of occurrence of each fish species during the study period were tabulated and presented in the form of pie chart.

3.5. Collection of water samples

Throughout the study period of ten months from Sep' 2016 to June' 2017, water samples were collected (following APHA, 2005) from five different localities [T₁ (Lat 08° 48' 48 N, Long 078° 09' 49E), T₂ (Lat 08° 48' 48 N, Long 078° 09' 52 E), T₃ (Lat 08° 48' 46 N, Long 078° 09' 50 E), T₄ (Lat 08° 48' 46 N Long 078° 09' 54 E), T₅ (Lat 08° 48' 50 N, Long 078° 09' 53 E)] in the sewage disposal site (Therespuram) and reference site (Vembar) in duplicates in clean and sterilized

plastic bottles except for dissolved oxygen and BOD, where BOD bottles were used. Dissolved oxygen was fixed on the spot; Temperature and pH were also measured on the spot. For various physico-chemical parameters, samples were analyzed in the laboratory as per Standard Methods (APHA, 2005). For estimation of heavy metals, samples were preserved by acidification with nitric acid.

3.6. Analysis of physico-chemical properties of water

Physico-chemical properties of water such as salinity, dissolved oxygen, biological oxygen demand (BOD), chemical oxygen demand (COD), total alkalinity and nutrients were analysed in the laboratory.

3.6.1. Temperature (°C)

The temperature of surface water was recorded in the study sites and reference site using a high quality Celsius thermometer of ± 0.5 °C accuracy. Two readings were observed and the mean was obtained with SD.

3.6.2. pH (Hydrogen ion concentration)

The hydrogen ion concentration (pH) of water samples were measured by a glass electrode and electrometer type pH meter, after taking necessary precautions in sampling and standardization.

3.6.3. Salinity

Salinity is a measure of total salt content of water. In the present work, chlorinity determination was made by using the standard titration method of the Mohr- Knudsen. The precipitable halide halogens in a 10 ml volume of water sample were determined by titration with silver nitrate solution using a chromate end point. Three to four drops of potassium chromate indicator were added to it. Silver nitrate

solution was standardized against 10 ml of seawater and the salinity value was estimated (Strickland and Parsons, 1972).

3.6.4. Dissolved Oxygen (DO)

For estimating the dissolved oxygen present in the seawater, the modified Winkler's (1883) method (Strickland and Parsons, 1968) was employed. In this method, the dissolved oxygen present (fixed during the time of sample collection) rapidly oxidizes an equivalent amount of divalent manganese to basic hydroxide of higher valency state. When the solution is acidified in the presence of iodide, the oxidized manganese reverts to the divalent state. Also iodine, equivalent to the original dissolved oxygen content of water sample is liberated, which is titrated against sodium thiosulphate solution with starch as the indicator. The values were expressed as mg.l^{-1} .

3.6.5. Alkalinity

The alkalinity of water can be determined by titrating the water samples with sulphuric acid of known concentration. Based on the stoichiometry of the reaction and volume of sulphuric acid needed to reach the end point, the concentration of alkalinity of water was calculated (APHA, 2005).

3.6.6. Total Dissolved Solids (TDS)

The water samples collected from various sampling sites were filtered through Whatman No. 4 filter paper. The clean filtrate was collected and exactly 100 ml of the filtrate was taken in a clean dry China dish of known weight (W_1 g). Evaporation (on water bath), drying (in an air oven at 110°C) and weighing, (after placing it in a

desiccators) were repeated to get concordant weight (W_2 g). From the difference in the initial and final weights, in mg, the total dissolved solids were calculated.

3.6.7. Total Suspended Solids

A water sample of 10 ml was filtered through pre-weighed Whatman No.4 filter paper. Later, the filter paper was dried in hot air oven and final weight of filter paper was noted. Then the difference between initial and final weight of filter paper multiplied with volume of sample gives the total suspended solids content of the water sample.

3.6.8. Chemical Oxygen Demand

Exactly 20 ml of water sample was taken in a 500 ml round bottomed (RB) flask. A known volume (10 ml) of potassium dichromate, 30 ml of concentrated sulphuric acid containing silver sulphate and mercuric sulphate were added to the RB flask. The reaction mixture was refluxed for 3 hr on a heating mantle. After cooling, the excess unreacted potassium dichromate was titrated against 0.25 N ferrous ammonium sulphate using ferroin indicator. A blank experiment was also carried out with distilled water.

3.6.9. Biological Oxygen Demand

Biological oxygen demand was calculated by incubation of water samples for five days at 20°C. Because of sewage water (high BOD value), diluted water was prepared by adding 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride per liter of distilled water. This diluted water was aerated half an hour to increase the DO content. Two sets of dilution factors (viz. 10 and 20) were tested to find out suitable dilution factor. Duplicate sets of BOD bottles were

incubated at 20°C for 5 days for each sample. The dissolved oxygen content of the above samples was calculated initially as well as after five days using Winkler's method. BOD value was calculated as follow:

Where,

$$\text{BOD}_5 \text{ (mg/l)} = (D_0 - D_5) - (DC_0 - DC_5) \times DF$$

D_0 = Initial dissolved oxygen

D_5 = Dissolved oxygen after five days

DC_0 = Initial dissolved oxygen of control group

DC_5 = Dissolved oxygen of control group after 5 days

DF = Dilution factor

3.6.10. Nutrients

The concentration of nutrients such as phosphate, silicate, ammonia, nitrate-N and nitrite-N were determined. For this, the samples were brought to laboratory after adding a few drops of chloroform as a preservative. In the laboratory, the samples were filtered by the Millipore filtering unit through Whatman GF/C filter paper (0.5 μ porosity).

3.6.10.1. Ammonia-N

Using the methodology of Solorzano (1969), ammonia estimation in seawater was carried out. To 25 ml of sample, 1 ml of phenol reagent, 1 ml of trisodium citrate and 1 ml of sodium hypochlorite reagent were added. It was then incubated at room temperature for 2 hr. The indo-phenol blue complex was formed. Blank and dilute NH_4Cl standards were analysed in the similar way for calibration. The absorbances were measured in a spectrophotometer at 630 nm.

3.6.10.2. Nitrate-N

Nitrate was analyzed by the method of Morris and Riley, (1963). The nitrate in the sample was reduced to nitrite almost completely by running the sample through a column containing copper coupled cadmium filings. Ammonium chloride was used as a buffer to maintain a stable pH during the passage of the sample through the reductor column (Grasshoff, 1976). The nitrite thus produced was measured by a spectrophotometer at 543 nm.

3.6.10.3. Nitrite-N

Nitrite was estimated by the method of Bend Schneider and Robinson (1952). Nitrite in the sample was allowed to react with sulphanilamide in an acid solution. The resulting diazo compound reacts with N-(1-naphthyl)-ethylene diamine dihydrochloride forming a diazo compound dye. Its light absorption was measured spectrophotometrically at 543 nm. Turbidity blanks were made by adding 0.5 ml of sulphanilamide solution to 25 ml of the sample.

3.6.10.4. Inorganic phosphate

The estimation of dissolved inorganic phosphate was carried out following the method of Murphy and Riley (1962). The samples were treated with a composite reagent containing molybdic acid and trivalent antimony. The resultant complex was reduced with ascorbic acid and the molybdenum blue solution formed was reduced with ascorbic acid and the molybdenum blue solution formed was measured spectrophotometrically at 885 nm. Turbidity blanks were made by adding 3.5 ml of the reagent without ascorbic acid to 25 ml of the samples.

3.6.10.5. Silicate (Ammonium Molybdate Method)

Silicate was estimated by adopting the method of Koroleff (1983). The estimation was based on the formation of a yellow silicomolybdic acid, when an acid sample was treated with a molybdate solution. The yellow complex thus produced could be reduced to a highly coloured blue complex with ascorbic acid and the intensity of the colour was measured spectrophotometrically at 815 nm.

3.7. GIS Mapping

Toposheet of Thoothukudi district was downloaded from the Texas University Website (www.lib.utexas.edu). It was geo-referenced using Q-GIS 1.8.0 Lisboa software. Then, the GPS coordinates of five different sampling stations in the sewage disposal site (Therespuram) were marked in the geo-referenced map. Later, the results of the water quality parameters were stored in GIS database. Spatial layers were created for each physico-chemical parameter and spread rate map was prepared using Arc-GIS software version 10.

3.8. Analysis of heavy metals in water samples

3.8.1. Collection of water samples for heavy metal analysis

The water samples were collected from 10 cm depth from the surface of water column from both the sewage polluted study site (Therespuram) and reference site (Vembar). The samples were collected in plastic bottles, before that the plastic containers were pre-cleaned with distilled water and soaked with 1:1 ratio of concentrated nitric acid and concentrated hydrochloric acid for 24 hrs to reduce potential source of metal contamination (APHA, 2005) and transported to the

laboratory immediately. The samples were kept in refrigerated condition until the analysis was carried on.

3.8.2. Analysis of heavy metals in water samples

For heavy metal analysis, one litre of water sample was collected. The water sample was filtered through 0.45 µm filter paper. Then, 500 ml was taken from the filtered water sample in a glass beaker and digested with 1:10 ratio of concentrated nitric acid and concentrated hydrochloric acid in hot plate. Digestion was carried out until the volume of solution turned to near dry condition. Then, the volume of the solution was made up to 20 ml with double distilled water. Later, the made up samples were transferred to plastic bottles and were analyzed for various metals in an Atomic Absorption Spectrophotometer (AAS).

3.8.3. Calculations

A calibration curve derived from the result obtained with the standard solutions was plotted. The concentration of metal was determined through the calibration curve. Standard stock solutions were prepared as per the method summarized in the working manual of AAS. The mean metal concentration in various samples were calculated by using the formula,

$$\text{Concentration of the metal in the sample} = \frac{\text{Conc. X Vol. of sample X Dil. factor}}{\text{Dry weight of the material (gm)}}$$

3.9. Analysis of heavy metals in fish tissues

3.9.1. Collection of fish specimens for heavy metal analysis

The fish specimens such as *Terapon puta* and *Arius maculatus* were collected in sterile polythene bags during sampling and stored in insulated container

filled with ice. Later, they were brought to laboratory immediately and stored in Ultra-Freezer at - 80° C.

3.9.2. Analysis of heavy metals in fish tissues

For heavy metal analysis, fish tissues like gill and muscle tissues were collected and stored in Ultra-Freezer at - 80° until the analysis of heavy metals. A known volume of fish tissue samples were taken and made into powder form by keeping the tissue samples in a hot air oven at 80 - 100° C overnight. Then the powdered fish tissue samples were digested with concentrated nitric acid and concentrated hydrochloric acid with ratio of 1:5 in hot plate. Then, the samples were filtered through Whatman No. 1 filter paper and final volume was made up to 20 ml with double distilled water.

3.9.3. Calculation of Bio accumulation Factor (BAF)

The bioaccumulation factor was calculated by following formula given by Devagi et al. (2008).

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{Metal concentration in tissue of an organism}}{\text{Metal concentration in water}} \times 100$$

The calculated BAF values are expressed in $\mu\text{g. l}^{-1}$.

3.10. Histology

3.10.1. Collection of tissue samples

The sample tissue like gill, kidney, liver, brain and muscle tissues were collected from *A. maculatus*, and *T. puta* collected from both the study site as well unpolluted reference site and the collected tissue samples were preserved in formalin (10% formaldehyde) and bouin's preservative.

3.10.2. Histology protocol

The sample tissues like gill, liver, kidney, brain and muscle (Formalin and Bouins preserved) were used for histological analysis. The fixed tissues were washed up in tap water. Then, the tissues were dehydrated with a series of upgraded alcohol solution (30% alcohol for 20 min; 50% for 20 min; 70% for 30 min; 90% for 30 min; 95% for 30 min; Absolute alcohol I for 45 min and Absolute alcohol II for 45 min) and the alcohol was cleared by a series of alcohol and xylene mixture [Alcohol (2) : Xylene (1) for 45 min; Alcohol (1) : Xylene (2) for 45 min; Xylene I for 1 hr and Xylene II for 1 hr] with using an Automatic Tissue Processor and a Cold Plate (Thermo Scientific). Thin sections (5 μ m sections) were taken from the processed tissues using microtome. The sections were floated in the tissue flotation bath maintained at 60°C and collected on clean slides applied with Mayer's albumin. The sections were fixed on the slides at 60°C using spirit lamp (Humason, 1972). The fixed slides were kept in different grades of xylene and alcohol as follows:

3.10.3. Staining of histological slides

Methodology for the haematoxylin and eosin (H&E) stain employed to assist in rapid histological processing

1.	Xylene I	:	5 min	13.	Eosin	:	10 sec
2.	Xylene II	:	5 min	14.	Washed in running tap water		
3.	Xylene II	:	3 min	15.	30% Alcohol	:	30 sec
4.	Alcohol I	:	3 min	16.	50% Alcohol	:	30 sec
5.	Alcohol I	:	3 min	17.	70% Alcohol	:	30 sec
6.	90% Alcohol	:	3 min	18.	90% methanol	:	2 min
7.	70% Alcohol	:	3 min	19.	Absolute Alcohol I	:	5 min
8.	50% Alcohol	:	3 min	20.	Absolute Alcohol II	:	10min
9.	25% Alcohol	:	3 min	21.	Xylene I	:	5 min
10.	Washed in running water	:	1 min	22.	Xylene II	:	5 min
11.	Hematoxylin	:	2 min	23.	Xylene I	:	Until cover
12.	Washed in running tap water			24.	Mounting using DPX mount		

Then, the mounted glass slides were observed under trinocular microscope with image processing system.

IV. RESULTS

In the present study, the impacts of sewage pollution on finfish and shellfish species composition, physico-chemical properties of seawater and histology of various organs of fishes like *Arius maculatus* and *Terapon puta* inhabiting sewage disposal site, Therespuram off Thoothukudi coast were investigated. Histopathological changes in gill, kidney, liver, brain and muscle tissues of the fishes under investigation were ascertained after comparing with the histology of respective organs of same fish species collected from relatively unpolluted reference site (Vembar).

4.1. Checklist of finfish, shellfish and sea urchin species encountered in sewage disposal site, Therespuram off Thoothukudi coast

Fishes were collected bimonthly from the sewage disposal site, Therespuram off Thoothukudi coast from September 2016 to June 2017 by employing gillnet. A total of 400 specimens of finfish, shellfish and sea urchin species were recorded during the study period. This includes 30 species of finfishes, 8 species of shellfishes and one species of sea urchin (Plate 4.1). The finfishes recorded were found to belong to seven orders with the dominance of the order Perciformes (9 families, 17 species), followed by Siluriformes (1 family, 6 species), Clupeiformes (2 families, 3 species). Each of the remaining orders like Pleuronectiformes, Tetradontiformes, Elopiformes and Mugiliformes were represented by one species. The shellfishes recorded included two species of shrimp (Family: Penaeidae), two species of crab (Family: Portunidae) and four species of gastropod (Family: Muricidae).

Table: 1. Checklist of finfish species collected from study site

Species Name	Common Name	Family	Order	No. Recorded	Size Range (Cm)	IUCN Status
<i>Arius jella</i> (Day,1877)	Blackfin Sea Catfish	Ariidae	Siluriformes	35	12.5 – 17.6	NE
<i>Arius maculatus</i> (Thunberg, 1792)	Spotted Catfish			124	12.2 – 30.5	NE
<i>Arius subrostratus</i> (Valenciennes, 1840)	Shovelnose Catfish			26	15.5 – 22.9	NE
<i>Plicofollis tenuispinis</i> (Day, 1877)	Thinspine sea catfish			25	13.5 – 24	NE
<i>Nemapteryx caelata</i> (Valenciennes, 1840)	Engraved Catfish			30	12.5 – 22	NE
<i>Arius arius</i> (F.Hamilton, 1822)	Threadfin Sea Catfish			20	11.2 – 25.2	LC
<i>Nematalosa nasus</i> (Bloch,1795)	Bloch's Gizzard Shad	Clupeidae	Clupeiformes	4	13 – 15.5	LC
<i>Thryssa mystax</i> (Bloch& Schneider, 1801)	Moustached Thryssa	Engraulidae		3	9.4 – 13.2	LC
<i>Thryssa setirostris</i> (Broussonet, 1782)	Longjaw Thryssa			3	11.2 – 13.5	LC
<i>Karalla daura</i> (Cuvier, 1829)	Goldstripe Ponyfish	Leiognathidae	Perciformes	6	7.2 – 10	NC
<i>Leiognathus equulus</i> (Forsskal,1775)	Common Ponyfish			3	5.5 – 8.9	LC
<i>Leiognathus brevirostris</i> (Valenciennes,1835)	Shortnose Ponyfish			2	5.6 - 6.8	NC
<i>Gerres erythrourus</i> (Bloch, 1824)	Deepbodied Mojarra	Gerridae		4	14.5 – 15.5	LC
<i>Epinephalus malabaricus</i> (Bloch& Schneider, 1801)	Malabar Grouper	Serranidae		4	8.5 – 9.5	NT
<i>Epinephalus tauvina</i> (Forsskal, 1775)	Greasy Grouper			2	12.5 – 14.5	DD
<i>Lutjanus fulvivlamma</i> (Forsskal, 1775)	Dory Snapper	Lutjanidae		7	10.5- 12.5	NE
<i>Lutjanus argentimaculatus</i> (Forsskal,1775)	Mangrove Snapper			1	21.5	NE
<i>Lutjanus johni</i> (Bloch,1792)	Johns Snapper			1	12.5	NE
<i>Scatophagus argus</i> (Linnaeus, 1766)	Spotted Scat	Scatophagidae		1	11.8	LC
<i>Terapan puta</i> (Cuvier, 1829)	Smallscale Banded Gunder	Teraponidae		50	6.5 – 11.5	NE
<i>Upeneus sulphureus</i> (Cuvier,1829)	Sulphur Goatfish	Mullidae		1	8.5	NE
<i>Parupeneus indicus</i> (Shaw,1803)	Indian Goatfish			1	10.5	NE
<i>Sillago sihama</i> (Forsskal, 1775)	Northern Whiting	Sillagnidae		1	13.5	NE
<i>Siganus lineatus</i> (Valenciennes, 1835)	Goldlined Spinefoot	Siganidae		1	18.5	NE
<i>Siganus canaliculatus</i> (Park, 1797)	Whitespotted Spinefoot			1	7.5	NE
<i>Siganus javus</i> (Linneaus, 1766)	Streaked Spinefoot			3	13.5	NE
<i>Cynoglossus bilineatus</i> (Lacepede, 1802)	Fourlined Tonguesole	Cynoglossidae	Pleuronectiformes	1	22.5	NE
<i>Arothron immaculatus</i> (Bloch & Schneider,1801)	Immaculate Puffer	Diodontidae	Tetradontiformes	1	12.5	NE
<i>Megalops cyprinoides</i> (Broussonet, 1782)	Indo Pacific Tarpon	Megalopidae	Elopiformes	1	14	NE
<i>Mugil cephalus</i> (Linneaus, 1758)	Flathead Grey Mullet	Mugilidae	Mugiliformes	35	12 – 27.5	NE

Table : 2. Checklist of shellfish and sea urchin species collected from study site

Table : 2. Checklist of shellfish and sea urchin species collected from study site						
Species Name	Common Name	Family	Order	No. Recorded	Size Range (In Cm)	IUCN Status
Shrimp						
<i>Fenneropenaeus indicus</i> (H. Milne-Edwards, 1837)	Indian White Prawn	Penaeidae	Decapoda	4	3.9 – 4.2 (CL)	NE
<i>Fenneropenaeus merguensis</i> (de Man, 1888)	Banana Prawn			5	2.8 – 3.4 (CL)	NE
Crab						
<i>Charybdis helleri</i> (A. Milne-Edwards, 1867)	Indo Pacific Swimming Crab (Spiny hand)	Portunidae	Decapoda	3	4.5 – 5.1 (CW)	NE
<i>Charybdis lucifera</i> (Fabricius, 1798)	Yellowish Brown Crab			3	3.5 – 4.2 (CW)	NE
Gastropod						
<i>Murex trapa</i> (Roding, 1798)	Rare- Spined Murex	Muricidae	Neogastropoda	2	6.5 – 7.8 (Ht.)	NE
<i>Murex tribulus</i> (Linnaeus, 1758)	Caltrop Murex			2	7.3 – 7.9 (Ht.)	NE
<i>Chicoreus virgineus</i> (Roding, 1798)	Virgin Murex			7	5.6 – 6.5 (Ht.)	NK
<i>Chicoreus capucinus</i> (Lamarck, 1822)	Mangrove Murex			4	5.7 – 7.2 (Ht.)	NK
Echinoderm						
<i>Salmacis virgulata</i> (L.Agassiz, 1846)	Sea Hedgehogs	Temnopluridae	Camarodonta	2	4.0 – 4.8 (Dia)	NE

Note: IUCN – International Union for Conservation of Nature and Natural Resources; NE – Not Evaluated; LC – Least Concern; NT – Near Threatened; DD – Data Deficient; NK – Not Known; CL – Carapace Length; CW – Carapace width; Ht. – Height; Dia – Diameter

In addition, only one sea urchin, species belonging to the family, Temnopleuridae under the order Camarodonta was recorded. The complete checklist of various finfish, shellfish and sea urchin species collected along with IUCN status are given in Tables 1 and 2.

4.2. Physico-chemical properties of seawater

Physico-chemical properties of seawater includes vital parameters such as temperature, salinity, pH, alkalinity, biological oxygen demand, chemical oxygen demand, total dissolved solids, total suspended solids, ammonia, nitrate, nitrite, phosphate and silicate. All these parameters are very significant as they influence both the chemical and biological environment. Hence, surface water sample was collected from all the five spots at sewage disposal site, Therespuram (T_1, T_2, T_3, T_4 and T_5) and reference site, Vembar (V) and various physico-chemical properties were analysed. The results of the analysis are as follows

4.2.1. Temperature

It is very important to measure temperature as it exerts effects on aquatic life, chemical reaction and rate and solubility of water. In the present study, temperature was found to range from 22.5 to 34°C. Maximum temperature ($34 \pm 0.5^\circ\text{C}$) was recorded at reference site during summer season and minimum temperature was at T_5 ($22.5 \pm 0.5^\circ\text{C}$) during monsoon season. Statistical analysis (one way ANOVA) of the result showed no significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. However, they were found to be positively correlated and the regression analysis showed that there

is a significant difference between the study site and reference site at 0.01% level (Figure 4.2.a).

4.2.2. Salinity

Salinity is one of the most important components of seawater that influences life forms. Salinity of surface water was found to range between 27 and 36 ppt. Maximum salinity (35 ± 1.06) was found at reference site (V) during summer season and minimum (27 ± 0.35) was at T_1 during monsoon season. Statistical analysis (one way ANOVA) of the result showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. Further, they were found to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.01% level (Figure 4.2.b).

4.2.3. Dissolved oxygen

Oxygen is an essential parameter which is used for metabolic process of living organism. Dissolved oxygen of the surface water was observed to range from 1.56 to 4.46 ppm. Maximum Dissolved oxygen (4.46 ± 0.1) was found at reference site (V) during pre-monsoon season and minimum (1.56 ± 0.1) was at T_1 during premonsoon season. One way ANOVA showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. Furthermore, they were found to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level (Figure 4.2.c).

4.2.4. pH

pH is an important parameter to measure the severity of acid and alkali present in water. The p^H was found to range from 6.78 to 7.85. Maximum p^H (7.8 ± 0.14) was recorded at reference site (V) during post monsoon season and minimum (6.78 ± 0.02) was at T_1 during post monsoon season. ANOVA (one way) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. In addition, they were found to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level (Figure 4.2.d).

4.2.5. Alkalinity

Alkalinity is an important factor used to measure capacity to neutralize acids. The alkalinity was found to range from 154 to 252 mg of CaCO_3/l . Maximum alkalinity (252 ± 1.85) was recorded at reference site (V) during summer season and minimum (154 ± 1.8) was at T_1 during post monsoon season. Statistical analysis (one way ANOVA) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. Further, they were found to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.01% level (Figure 4.2.e).

4.2.6. Total Dissolved Solids (TDS)

The TDS fluctuated between 29.12 and 80.25 ppt. The highest value was (80.25 ± 3.44) recorded at T_1 during monsoon season and the lowest value was (29.12 ± 0.69) at reference site during summer season. ANOVA (one way) showed significant difference between any two study sites and also between anyone of the

study site and reference site at 0.05% level. Besides, they were noted to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.01% level (Figure 4.2.f).

4.2.7. Total Suspended Solids (TSS)

Total suspended solids are solids in water that include a wide variety of material, such as silt, decaying plant and animal matters, industrial wastes and sewage. River runoff effluents from coastal industries and sewage discharge from settlements contribute suspended solids into coastal areas. The TSS was found to range from 0.8 to 11.8 ppt. Maximum TSS (11.8 ± 0.06) was recorded at T₁ during monsoon season and minimum (0.75 ± 0.08) was at reference site (V) during post monsoon season. ANOVA (one way) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. Likewise, they were found to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level (Figure 4.2.g).

4.2.8. Biological Oxygen Demand (BOD)

BOD is the amount of dissolved oxygen demanded by aerobic biological organism to break down organic material present in a given water sample at certain temperature over a specific time period. The lowest value of BOD in the present study was observed to be 1.82 ± 0.15 ppm at reference site (V) during monsoon season and the highest value was 62 ± 1.41 ppm from study site T₁ during pre-monsoon season. Statistical analysis (one way ANOVA) of the result showed significant difference between any two study sites and also between anyone of the

study site and reference site at 0.05% level. Moreover, they were observed to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level (Figure 4.2.h).

4.2.9. Chemical Oxygen Demand (COD)

COD is a measure of the oxygen demand created by toxic organic and inorganic compounds and also by biodegradable substances. The COD was estimated to range from 4.52 to 152 ppm. The maximum COD (152 ± 6.08) was recorded at T₁ during pre - monsoon season and minimum (4.52 ± 0.15) was at reference site (V) during monsoon season. ANOVA (one way) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. Moreover, they were found to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level (Figure 4.2.i).

4.2.10. Ammonia - Nitrogen

Ammonia concentration was found to range from 0.3751 to 1.536 $\mu\text{g atm NH}_3\text{-N/ ml}$. Maximum ammonia concentration (1.536 ± 0.14) was recorded at T₂ during pre-monsoon season and minimum (0.3751 ± 0.08) was at reference site (V) during monsoon season. ANOVA (one way) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. Furthermore, they were found to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level (Figure 4.2.j).

4.2.11. Nitrate-Nitrogen (NO₃-N)

Nitrate concentration was found to range from 0.1752 to 1.2117 $\mu\text{g atm NO}_3\text{-N/ ml}$. The maximum nitrate concentration (1.2117 ± 0.08) was recorded at T₁ during post- monsoon season and minimum (0.1752 ± 0.02) was recorded at reference site (V) during pre monsoon season. Statistical analysis (one way ANOVA) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. In addition, they were observed to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.01% level (Figure 4.2.k).

4.2.12. Nitrite-Nitrogen (NO₂-N)

Nitrite concentration varied from 0.047 to 1.567 $\mu\text{g atm NO}_2\text{-N/ ml}$. The maximum nitrite concentration (1.567 ± 0.03) was recorded at T₃ during pre monsoon season and minimum (0.047 ± 0.02) was recorded at reference site (V) during monsoon season. ANOVA (one way) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. Besides, they were noted to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level (Figure 4.2.l).

4.2.13. Phosphate (PO₄-P)

Phosphate is present in water by addition of fertilizer, pesticides, animal waste and industrial waters. The value of phosphate varied between a minimum of 1.098 $\mu\text{g atm PO}_4\text{-P/ml}$ at reference site (V) during summer season to a maximum of 11.79 $\mu\text{g atm PO}_4\text{-P/ml}$ at T₂ during pre-monsoon season. Statistical analysis (one

way ANOVA) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. In addition, they were observed to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.01% level (Figure 4.2.m).

4.2.14. Silicate ($\text{SiO}_4\text{-Si}$)

Silicate concentration was determined to range from 2.129 to 10.16 $\mu\text{g atm SiO}_4\text{-Si/ml}$. The maximum silicate concentration (7.528 ± 0.56) was recorded at T_1 during summer season and minimum (2.129 ± 0.85) was recorded at reference site (V) during the same season. ANOVA (one way) showed significant difference between any two study sites and also between anyone of the study site and reference site at 0.05% level. Besides, they were noted to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level (Figure 4.2.n).

4.3. GIS Mapping

The various water quality parameter readings were stored in GIS database. Then, spread rate map was prepared for each of the physico-chemical parameter studied with the aid of Arc-GIS (version 10). It was prepared in relation to various physico-chemical parameters of water collected from sewage polluted disposal site, Therespuram, off Thoothukudi coast for monsoon season (Plate 4.9, 4.10 and 4.11) and summer season (Plate 4.12, 4.13 and 4.14) separately.

4.4. Heavy metal analysis

4.4.1. Seawater samples

Heavy metals such as copper, cadmium, lead and nickel were estimated in water samples collected from exact point of sewage disposal at Therespuram (T_1), off Thoothukudi coast and reference site (Vembar) during summer season. Statistical analysis of (One way ANOVA) of the concentration of all the heavy metals in water samples of study site, T_1 and reference site (V) showed significant difference between the study site and reference site at 0.05% level. The results are as below (Figure 4.3.a):

4.4.1.1. Copper

The concentration of copper in water samples varied from a minimum of 2.24 ppm at reference site (V) and to a maximum of 3.04 at study site (T_1).

4.4.1.2. Cadmium

The concentration of cadmium fluctuated between 0.0148 and 0.14 ppm. Maximum cadmium concentration (0.14 ppm) was found in water samples of study site (T_1) while it was minimum (0.0148 ppm) at reference site (V).

4.4.1.3. Lead

The concentration of lead was found to range from 1.9 to 2.19 ppm. Maximum concentration of lead (2.9 ppm) was recorded in water samples of study site (T_1), whereas it was minimum (1.9 ppm) at reference site (V).

4.4.1.4. Nickel

The concentration of nickel in water samples was found to range from 2.4 to 2.96 ppm. Maximum concentration of nickel (2.96 ppm) was found in water samples of study site (T_1), whereas it was minimum (2.4 ppm) at reference site (V).

4.4.2. Tissues

4.4.2.1. Copper

The concentration of copper ranged from 0.12 to 1.38 ppm. The maximum concentration (1.382 ppm) was recorded in gill tissues of *T. puta* collected from sewage polluted study site and minimum concentration (0.126 ppm) was found in muscle tissues of *T. puta* collected from reference site (V). It was found to be intermediate in both gill and muscle tissues of *A. maculatus* (Figure 4.3.b).

4.4.2.2. Cadmium

The concentration of cadmium ranged from 0.013 to 0.88 ppm. The maximum concentration (0.88 ppm) was found in gill tissues of *T. puta* collected from study site and minimum (0.013 ppm) was found in muscle tissues of *T. puta* collected from reference site. It was found to be intermediate in both gill and muscle tissues of *A. maculatus* (Figure 4.3.b).

4.4.2.3. Lead

The concentration of lead was measured to range from 0.022 to 1.63 ppm. The maximum concentration (1.66 ppm) was found in gill tissues of *A. maculatus* collected from study site and minimum concentration (0.022 ppm) was found in muscle of *T. puta* collected from reference site (V). It was intermediate in muscle tissues of *Arius maculatus* and gill tissues of *T. puta* (Figure 4.3.b).

4.4.2.4. Nickel

The concentration of nickel in tissue varied between 0.017 and 1.38 ppm. The maximum concentration (1.38 ppm) was found in gill tissues of *A. maculatus* collected from study site and minimum concentration (0.017 ppm) was found in muscle of *A. maculatus* collected from reference site (V). It was found to be intermediate in both gill and muscle tissues of *T. puta* (Figure 4.3.b).

4.4.3. Bioaccumulation of heavy metals in tissues

Bioaccumulation of heavy metal was estimated in terms of bioaccumulation factor for both the fish species collected from study site and reference site (Figure 4.3.c and 4.3.d).

4.4.3.1. Copper

The bioaccumulation factor (BAF) for copper was estimated to be 5.16 to 45.46 %. The highest BAF (45.16%) was recorded from gill tissues of *T. puta* collected from study site. The lowest BAF (5.16%) was from muscle tissues of *T. puta* collected from reference site (V). It was intermediate in gill and muscle tissues of *A. maculatus*.

4.4.3.2. Cadmium

The bioaccumulation factor (BAF) for cadmium was estimated to range from 0.27 to 6.29 times of cadmium present in water. The highest BAF (628.57%) was recorded from gill tissues of *T. puta* collected from study site. The lowest BAF (27%) was from muscle tissues of *T. puta* collected from reference site (V). It was intermediate in gill and muscle tissues of *A. maculatus*.

4.4.3.3. Lead

The bioaccumulation factor for lead was estimated to be 1.11 to 74.77%. The highest BAF (74.77%) was recorded from gill tissues of *A. maculatus* collected from study site. The lowest BAF (1.11%) was found in muscle tissues of *T. puta* collected from reference site. It was intermediate in muscle tissues of *A. maculatus* and gill tissues of *T. puta*.

4.4.3.4. Nickel

The bioaccumulation factor (BAF) for nickel was estimated to be 0.702 to 46.62%. The highest BAF (46.62%) was recorded from gill tissues of *A. maculatus* collected from study site. The lowest BAF (0.702%) was noted in muscle tissues of *A. maculatus* collected from reference site. It was intermediate in gill and muscle tissues of *T. puta*.

4.5. Histology

In this study, the histology of gill, liver, kidney, brain and muscle tissues of *A. maculatus* and *T. puta* collected from sewage polluted study site and relatively unpolluted reference site were observed under light microscope. Then, histology of various organs of these two species collected from sewage disposal site was compared with the histology of respective organs of the same species collected from relatively unpolluted reference site and histopathological changes were marked.

4.5.1. Histology of fishes collected from reference site

4.5.1.1. Histology of *Arius maculatus*

4.5.1.1.1. Gill

Gill sections from *A. maculatus* collected from reference site showed normal histoarchitecture. Normal blood vessels, primary gill lamellae and secondary gill lamellae were observed (Plate 4.15.a).

4.5.1.1.2. Liver

The histological analysis of liver tissue of *A. maculatus* collected from reference site showed normal structure with typical hepatic tissue, hepatocyte with nucleus, blood sinusoid, kupffer cells and pycnotic nucleus (Plate 4.15.b).

4.5.1.1.3. Kidney

The histological investigation of kidney tissues of *A. maculatus* collected from reference site showed normal histoarchitecture. Kidney was characterized by well-built haemopoietic tissue, typical renal tubule and glomerulus with clear Bowman's capsule (Plate 4.15.c).

4.5.1.1.4. Brain

Brain was characterized by presence of granular layer, Purkinje cell dendrites and Purkinje cell nucleus (Plate 4.15.d).

4.5.1.1.5. Muscle

The histological study of muscle tissue of the control fish, *A. maculatus* showed the presence of normal myomere with equally spaced muscle bundles which indicate that the fish was in unstressed condition (Plate 4.15.e).

4.5.1.2. Histology of *Terapon puta*

4.5.1.2.1. Gill

Histology of gill of *T. puta* was observed to resemble gill sections of *A. maculatus*. It showed normal histoarchitecture with normal blood vessels, primary gill lamellae and secondary gill lamellae were observed (Plate 4.16.a).

4.5.1.2.2. Liver

The histological examination of liver tissue of *T. puta* caught from reference site (V) showed typical hepatocytes, hepatocyte with nucleus, blood sinusoid, kupffer cell, and pycnotic nucleus (Plate 4.16.b).

4.5.1.2.3. Kidney

The histological examination of kidney tissue of *T. puta* caught from reference site (V) showed typical renal tubules, Bowman's capsule, glomerulus, and haemopoietic tissues (Plate 4.16.c).

4.5.1.2.4. Brain

Histology of brain of *T. puta* was observed to look like the brain sections of *A. maculatus*. The histological examination showed typical Purkinje cell dendrites, Purkinje cell nucleus and pyramidal cell (Plate 4.16.d).

4.5.1.2.5. Muscle

The histological examination of muscle tissue of *T. puta* caught from reference site (V) showed structure of normal muscle bundles like *A. maculatus* (Plate 4.16.e).

4.5.2. Histopathology of fishes collected from sewage disposal site

Histological sections of organs such as gill, liver, kidney, brain, and muscle tissues were prepared from *A. maculatus* and *T. puta* collected from sewage disposal site bimonthly during Sep' 2016 to June' 2017. The observations of the histological sections observed under light microscope revealed several tissue specific alterations in common in all the organs studied. However, the intensity of the alterations was observed to be the highest in the fish specimens collected during the summer season and the lowest in fish specimen collected during monsoon season. Hence, presently, histopathology of the various organs of *A. maculatus* and *T. puta* collected from the sewage disposal site during monsoon season and summer season alone are presented and compared.

4.5.2.1. Histopathology of *Arius maculatus*

4.5.2.1.1. Gill

The histological examination of gill tissues of *A. maculatus* collected from the study site during monsoon season showed several structural alterations. Marked variations like hyperplasia, vacuolation, deformation of cartilage core, bubbling of gill filament, epithelial lifting and lamellar fusion were noticed (Plate 4.17.a). The histological examination of gill tissues of *A. maculatus* collected from the study site during summer season showed specific structural alterations like secondary lamellar damage, shorter secondary lamellae and erosion of secondary lamellae in addition the alterations occurred during monsoon season (Plate 4.19.a).

4.5.2.1.2. Liver

The histological examination of liver sections of *A. maculatus* collected from the study site during monsoon season showed abnormalities like blood congestion, cytoplasmic vacuolation, rupture of hepatocytes, vacuolation and degenerated nucleus. (Plate 4.17.b), whereas in summer season, besides the above-mentioned alterations, nucleus disorganization was also noticed (Plate 4.19.b).

4.5.2.1.3. Kidney

The histological analysis of kidney of *A. maculatus* collected during monsoon showed blood congestion, cytoplasmic vacuolation, and vacuolation (Plate 4.17.c), while kidney sections of the said species collected during summer season showed marked variations like increased periglomerular space, degenerated glomerulus and increased peritubular space, melanomacrophage in addition to the alterations occurred during monsoon season (Plate 4.19.c)

4.5.2.1.4. Brain

The histological examination of brain of *A. maculatus* collected during monsoon showed profound alterations like enlarged pyramidal cell, vacuolation, binucleated nucleus, ruptured nucleus, and haemorrhage (Plate 4.17.d) during summer season, histological sections showed similar alterations but with high intensity (Plate 4.19.d).

4.5.2.1.5. Muscle

The histopathology of muscle tissue of *A. maculatus* collected during monsoon showed some remarkable changes like splitting muscle bundles and hyaline degeneration (Plate 4.17.e) whereas during summer season degeneration of muscle bundles and vacuolar degeneration were also observed along with above-mentioned two changes (Plate 4.19.e).

4.5.2.2. Histopathology of *Terapon puta*

4.5.2.2.1. Gill

The histological examination of gill of *T. puta* collected during monsoon season exhibited the abnormalities like showed hyperplasia, mucous exordial, epithelial lifting and lamellar fusion (Plate 4.18.a). However, during summer season,

in addition to epithelial lifting and lamellar fusion, abnormalities like epithelial rupture, haemorrhage, vacuolation and deformation of cartilage core were also noticed (Plate 4.20.a)

4.5.2.2.2. Liver

The histological examination of liver sections of *T. puta* showed increased Kupffer cell, increased pycnotic nucleus, vacuolation, ruptured nucleus, and melanomacrophage (Plate 4.18.b). Similar kinds of alterations were noticed in liver sections collected during summer season but with severe rupture of hepatocytes (Plate 4.20.b).

4.5.2.2.3. Kidney

The histological examination of liver sections of *T. puta* collected during monsoon showed blood congestion, increased periglomerular space, cytoplasmic vacuolation, rupture of Bowman's capsule, increased peritubular space and melanomacrophage (Plate 4.18.c). Similar alterations were noticed in kidney sections collected during summer season but with enlarged melanomacrophages (Plate 4.20.c).

4.5.2.2.4. Brain

The histological examination of brain sections of *T. puta* collected during monsoon season exhibited enlarged pyramidal cell, ruptured nucleus and binucleated nucleus (Plate 4.18.d). However, during summer season, vacuolation, degenerated nucleus and hemorrhage were also noticed in addition to the alterations occurred during monsoon season (Plate 4.20.d).

4.5.2.2.5. Muscle

The histological examination of muscle sections of *T. puta* collected during monsoon season exhibited splitting muscle bundles, hyaline degeneration, and degeneration of muscle bundles (Plate 4.18.e). During summer season, granuloma and severe muscle bundle degeneration were also noticed along with the alterations occurred during monsoon season (Plate 4.20.e).

V. DISCUSSION

Globally sewage remains the largest source of contamination by volume of the marine environment (GESAMAP, 2001) and coastal sewage discharges have increased dramatically in the past three decades (Isalm and Tanaka, 2004). Hence, the present investigation was undertaken to know the effects of sewage pollution on finfish and shellfish composition, physico-chemical properties of seawater and histology of various organs of fishes like *Arius maculatus* and *Terapon puta* collected from sewage disposal site at Therespuram off Thoothukudi coast.

5.1. Finfish and shellfish faunal composition

Periodic fish sampling done by gillnetting from Sep' 2016 to June' 2017 revealed the finfish and shellfish faunal composition of sewage disposal site at Therespuram off Thoothukudi, Southeast coast India. The survey showed presence of 400 specimens of 30 finfish species belonging to 7 orders, 16 families of the class Actinopterygii; 2 shrimp species of the family Penaeidae and 2 crab species of the family Portunidae under the order Decapoda and one species of sea urchin belonging to the order Camarodonta and family Temnopluridae. Though the order, Perciformes was found to dominate with 9 families and 17 species, the order Siluriformes was observed to dominate with most number of specimens collected (65 %), followed by Perciformes with 22.25%. Among the species, *A. maculatus* was found to dominate with 31% of specimens collected, followed by *T. puta* with 12.5% (Table 1, Figures 4.1 a, 4.1 b, and 4.1 c). Among the shellfishes, the family, Muricidae was observed to dominate with 4 species (15 specimens), followed by 2

shrimp species (9 specimens), and 2 crab species (6 specimens) (Table 2; Fig 4.1.d).

Fishes as a group, apart from its economic value, from a biodiversity viewpoint, has the highest species diversity among all vertebrate taxa. It is believed that out of 61,259 species of vertebrate recognized world over, 32,300 are fish species; of which 15,170 are freshwater while 16,764 are marine (William *et al.*, 2010). The estimated number of 2,443 species of marine fish recognized from India is taxonomically distributed in 230 families (Venkatraman and Sivaperuman, 2015). Sekher (2013) reported a total of 1,121 species of fishes belonging to 35 orders, 166 families and 539 genera from east coast of India. A total of 1,182 fish species belonging to 476 genera, 144 families and 39 orders have been recorded from the Gulf of Mannar Ecosystem (Joshi *et al.*, 2016). However, in the present study, only few finfish and shellfish species have been encountered which might be due to sewage pollution in the study site as suggested by Plafkin *et al.* (1989) who opined that a community becomes more dissimilar as the stress increases and accordingly species diversity decreases with poor quality. Communities dominated by relatively few species indicate that the environment is under stress. Our present study is in agreement with Plafkin *et al.* (1989) as only 39 finfish and shellfish species (including sea urchin) have been collected with the dominance of bottom dwelling catfishes belonging to the family Ariidae with 65%. Further, the diversity of shellfish was very poor in the study site which might be either due to slow moving nature of these animals making them avoid the polluted environment or due to inability of gillnet in catching these shellfishes. From the observation of the size of the

specimens recorded, it is learnt that breeding might have taken place in the polluted site in some of the species recorded, especially *A. maculatus*.

Pastorok and Bilyard (1985) undertook a study on impact of sewage pollution on coral reef communities, and reported that sewage pollution causes major changes in species composition and abundance. Our study is in conformity to the study carried out by Azzuro *et al.* (2011) who reported that sewage caused significant changes in fish assemblage and individual species level in Mediterranean waters. Further, they added that fish assemblage structures were different between control and sewage disposal site whereas, total abundance of fish were higher. The responses of individual species to sewage pollution were related to an anomalous increase of small opportunistic species and also significant changes of the fish size distribution were also detected for several species. Shrivastav and Singh (2016) reported only 21 fish species in the water bodies contaminated with fly ash with the dominance of the order, Cypriniformes with 14 species. As suggested by Shrivastav and Singh (2016), the present study may be helpful for developing strategies for the conservation of fish species in the study area.

5.2. Physico-chemical properties of seawater

Hydrographic parameters are vital for the normal growth of biota in a water ecosystem. The marine hydrography is much complicated due to the dynamic nature of this ecosystem. Changes in the hydrographical parameters such as salinity, dissolved oxygen, dissolved carbon dioxide; nutrients and so on affect the activities and growth of the organisms in the ecosystem (Sridhar *et al.*, 2008). Hydrographic parameters of different sites of Gulf of Mannar have been reported by several

authors. Yogesh Kumar (2010) investigated the impacts of industrial pollution on the coral islands of Thoothukudi district in Gulf of Mannar. Vanmathi (1999) and Subbulakshmi (2005) conducted detailed investigations on the pollution of Tuticorin coastal waters. The general coastal water quality standards (swimming and aquatic life) for several parameters are specified in Table 3 (Moore, 1991; National Environmental Board Report, 1994).

Table 3. General coastal water quality standards (Swimming and Aquatic Life)

Parameters	Standards
pH	7.8 – 8.3
Temperature	30
Total suspended sediments (TSS)	25 mgL ⁻¹ or less
Dissolved oxygen	4 mgL ⁻¹ or more
Biochemical Oxygen Demand (BOD ₅)	30 mgL ⁻¹ or less
Chemical Oxygen Demand (COD)	250 mgL ⁻¹ or less
Nitrate	10 mgL ⁻¹ or less
Nitrite	10 mgL ⁻¹ or less
Phosphorous as Phosphate PO ₄	0.1 mgL ⁻¹ or less
Cadmium	0.01 mgL ⁻¹
Lead	0.1 mgL ⁻¹
Copper	0.02 mgL ⁻¹
Nickel	0.01 mgL ⁻¹

In the present study, water temperature fluctuated widely between 22.5 to 34°C. Minimum temperature (22.5±0.5°C) recorded at T₅ might be due to the discharge of sewage with freshwater run-off during monsoon season. The low temperature might have been responsible for the low finfish and shellfish diversity. This is in conformity to the study conducted by Gopinathan et al., (1991) who reported that tropical aquatic organisms including shell and fin fishes grow very well at water temperature ranging between 25 and 30. However, it should be noted that temperature at the study site is within the permissible limit of the International standards.

Total alkalinity measures the capacity of the water to neutralize acids, testing the alkalinity of water is important to determine seawater ability to neutralize acidic pollution from wastewater. According to US Environmental Protection Agency, the total alkalinity of seawater ranges from 80 - 116 mg/l. in present study the alkalinity of study site and reference site is higher than the standard. But study having less alkalinity compared to reference site that is because of domestic water discharge.

The salinity of coastal and offshore environment is influenced by a number of environmental factors such as runoff, precipitation evaporation, surface current patterns, and upwelling (Bruckner and Burrows, 2005). In the present study, salinity varied between 27 and 36 ppt. A very marked decline in salinity values were observed during the monsoon season at the sewage disposal site, T₁. The low value of salinity during the monsoon season noted in the present study may be due to the entry of fresh water into the sea due to monsoon (Kumaraguru and Jayakumar, 1998; Asha et al., 2009). Low salinity and wide salinity fluctuation might not have favoured the growth of shellfishes in the sewage disposal site. This is in agreement with the study conducted by Yogesh Kumar (2010) who opined that wide salinity fluctuations and factors that increase or decrease salinity levels can have detrimental impacts on benthic reef building corals.

The dissolved oxygen in water and available to aquatic organisms, one of the most important indicators of the condition of a water body; concentrations below 5 mg/l are stressful and may be lethal to many fish and other species (Bruckner and Burrows, 2005). Dissolved oxygen levels in seawater column are related to several other factors such as temperature, salinity, wave action, amount of plankton present,

pollution and the influence of including external water masses (Sivasamy, 1990). The dissolved oxygen is known to decide the suitability of aquatic ecosystems to support the survival and growth of aquatic organisms. In the present study, dissolved oxygen concentration ranged between 1.56 to 4.46 ppm. It should be noted that dissolved oxygen at the study site is not within the permissible limit of the International standards. The low dissolved oxygen recorded at sewage disposal site, T₁ might have been the reason for the low fish diversity. The present study is in compliance with the study of Chandraprakash and Reddy (1999) who reported that dissolved oxygen concentrations greater than 3.5 ml/l were always associated with rich fish populations and species diversity.

Atkins, (1992) established pH of the water as indispensable factors for all hydrographical studies. The pH of sea water generally ranges from 7.5 to 8.4, and is slightly alkaline (Nair and Thampy, 1980). The high buffering activity of sea water often checks wide variations of pH in coastal waters (Martin, 1970). In the present study the pH of the seawater varied from 6.78 to 7.85. The minimum pH value was recorded at the sewage disposal site T₁ during monsoon season. The minimum pH value recorded during the monsoon season could be due to entry of fresh water (Alongi et al., 1989; Sridhar et al., 2008).

In the present study, alkalinity of water fluctuated widely between 154 and 252 mg of CaCO₃/l in the study sites which indicate that the study sites have the buffering capacity to neutralize the acidic pH. Seawater typically is very salty (TDS > 35,000 mg/l). In the present study, TDS fluctuated from 29.12 to 80.25 ppt. The highest value was recorded at T₁ during monsoon season. The materials

brought out by the sewage during the monsoon season might have contributed to the high TDS in the study site.

The Total Suspended Solids was found to range from 8 to 11.8 ppt (i.e., 800 to 11,800 ppm). The maximum value was recorded at T₁ during monsoon season. It is worth-mentioning here that TDS at the study site is not within the permissible limit of the International standards. The Biochemical Oxygen Demand (BOD) was estimated to be from 1.82 to 62 ppm. The highest value recorded at T₁ during pre-monsoon season is not within the permissible limit of the International standards. The high TDS and BOD might be attributed to low fish diversity in the study area. However, the Chemical Oxygen Demand (COD) was found to be well within the limit of the International standards i.e., from 4.52 to 152 ppm.

The nutrient content of the marine ecosystem is determined by their concentrations in the riverine and coastal water source and the properties of each in the mixture (Smayda, 1983). The dissolved nutrients are indispensable constituents for primary production. Of these, nitrate and phosphate enhance primary productivity. Both are necessary for the survival of autotrophy, yet they exist in very small concentrations in water (Valiela, 1984). Steeman and Neilson (1959) considered nutrient replenishment as the single most important factors in controlling phytoplankton growth in the tropics. Irrespective of man's influence, the concentration of the nutrients like phosphate and nitrate are characteristically higher in river water and lead to a general enhancement of nutrients in coastal and estuarine water influenced by land drainage (Raymont, 1980). In the present study, normal range of phosphate (0.14µg.l⁻¹ to 1.5µg.l⁻¹) were recorded in all stations

during all seasons which is very close to the previous reports evolved from this area (Kumaraguru and Jeyakumar, 1998). In the present study, nitrite and nitrate were well within the permissible limit of the International standards described in Table 3. Sources of nitrate and nitrite in the study area are mainly due to the domestic sewage, natural run-off, agricultural waste and some industrial wastes (Smayda, 1983). Wide range in nitrate content has also been reported by Mohamed et al., (2009) in Gulf of Mannar coast. The high concentration of nitrate in the present study may be due to heavy monsoon rains which brought in large amount of nutrients into the marine ecosystem through the river such as Mookiyour, Vembar and Vippar river and the major Thoothukudi sewage waste dumping channel the Buckle Canal. Nitrite usually occurs at much lower concentration than the nitrate. Nitrite has been reported to be accumulated to appreciable levels only in regions of low oxygen tension (Morris, 1980).

However, the Phosphate concentration was observed to range from a minimum of 1.098 $\mu\text{g atm PO}_4\text{-P/ml}$ at reference site (V) during summer season to a maximum of 11.79 $\mu\text{g atm PO}_4\text{-P/ml}$ at T₂ during pre-monsoon season. These values were not within the permissible limit of the International standards. In the present study, dissolved inorganic phosphate was found to be in high concentration during pre-monsoon in the study site as reported in Thoothukudi and Vembar group of islands by Gopinathan and Rodrigo (1991); Kumaraguru and Jayakumar (1998); Palanichamy et al. (1995); Asha et al. (2009). Inorganic phosphate concentration is a useful index of the state of eutrophication of water bodies (Ketchum, 1967). Water receiving raw or untreated sewage, agriculture drainage and certain industrial waste

usually contain significant concentration of phosphate. Phosphorus is as soluble reactive and dissolved organic phosphorus and polyphosphate (Rajashree et al., 1993). Silicate concentration was also measured to be high in the study site, which may result in eutrophication. Silicate is a nutrient essential for the growth of phytoplankton. In the present study, the silicate concentration was observed to range from a minimum of 2.129 $\mu\text{g atm SiO}_4\text{-Si/ml}$ at reference site (V) during summer season to a maximum of 10.16 $\mu\text{g atm SiO}_4\text{-Si/ml}$ at T₁ during summer season.

The observations of the water quality parameters are in conformity to the result reported by Niroshana et al. (2013) from Purnawella fishery harbour of Srilanka, Chandar and Kumar (2012) and Balakrishnan et al. (2015) from Thoothukudi coast.

5.3. GIS mapping

Geographic Information System (GIS), a high performance computer based tool is playing a critical role in water resource management and pollution study. Spatial variability map of different seawater quality parameters was generated using interpolation operation in the Arc-GIS software (Plate 4.9 – 4.14) (Igboekwe, M.U., and Akankpo., 2011).

Despite several efforts in the past three decades, the procedures using satellite imagery to measure surface water quality have not been adopted on a routine basis (Brezonik et al, 2007). Further, no such attempt for extent of sewage pollution based on water quality and image analysis has been reported in the literature. Hence, in the present study, spread rate map was prepared in relation to

various physico-chemical parameters of water collected from sewage polluted disposal site of Therespuram off Thoothukudi coast by using Arc-GIS. A good correlation exists between some of the pollution indicators (total dissolved solids, TDS and nutrients). The results of spatial variability maps of different seawater quality parameters indicate an increase in the percentages of pollution towards shore site as reported by Igboekwe, and Akankpo (2011). The map covered area of about one sq.km near the sewage disposal site. The map indicates that there was a gradual increase of nutrients towards the coastal side where the sewage is disposed into the sea.

The sewage pollution spread rate map was prepared for summer and monsoon separately. From the sewage spread rate map it is learnt that there is a gradual reduction in nutrients towards the sea. During monsoon month which might be due to a rainfall runoff which diluted sewage polluted water. Rainfall resulted in gradual mixing that produced a clear demarked hotspot map. In summer season due to less rainfall and uneven mixing the hotspot of higher concentration was located here and there in the present study.

A similar kind of study was undertaken by Igboekwe, and Akankpo (2011) for mapping groundwater quality in Uyo, Nigeria, Their study suggested that Geographic Information System (GIS) software is very useful in the analysis of distribution of groundwater pollution. Various human activities generate wastes which have high in organic and inorganic contents which are directly or indirectly disposed on land without any pretreatment, thereby affecting the groundwater water quality.

Asad et al. (2011) studied the pollution prevention study using Remote Sensing and GIS in Visakhapatnam district, Andhra Pradesh. They used Visual Interpretation technique to prepare different thematic maps like land use/land cover, base map, village information, drainage maps. GIS overlay analysis derived maps like surface water Table, surface water use, surface water quality, was carried out to find out the pollution loads in the study area.

Chandar and Kumar (2012) also analysed the marine pollution in Tuticorin coastal areas using remote sensing and GIS. In their study they analysed the spread rate and hotspot with the aid of various physical, chemical, biological parameters of water. They opined that Tuticorin Thermal Power Station (TTPS) and Threspuram has high rate of pollution. In Therespuram, the pollution is due to anthropogenic activity. Further, it was advised to link the sewage into deep sea at a minimum depth of 20 m after the primary treatment of waste water.

Vijay et al. (2015) studied the extent of sewage pollution in coastal environment of Mumbai by using object based image analysis. The study advocated that the potential application of object based image classification in identification and assessment of the extent of sewage pollution in coastal environment for better planning and management. In this regard, the map generated in the present study would be helpful for monitoring pollution in the sewage disposal sites (Plates 4.9 – 4.14).

5.4. Heavy metal analysis in seawater

It should be noted that the values of heavy metals such as copper, cadmium, lead and nickel recorded from the study site during the study period were not within

the permissible limit of the international standards. However, the concentration of same heavy metals were within the permissible limit of International standards in reference site (Vembar) except lead which might be due to berthing of fishing boats along the shore.

Standard levels of metals in fish (1 g wet weight) described by various organization

Organization / Country	Cd	Cu	Pb
European Community	0.05	-	0.2
England	0.2	20	2.0
FAO	-	30	0.5
FAO / WHO Limits	0.5	30	0.5

EC- European country; FAO – Food and Agriculture Organization; FAO/WHO – Food and Agriculture Organization / World Health Organization.

The concentration of copper in water samples varied from a minimum of 2.24 ppm at reference site (V) and to a maximum of 3.04 at study site (T₁). In the present study the observed copper level in the seawater might be due to the effluents discharged from the industries of Thoothukudi, urban runoff, harbour wastes and the antifouling paints painted on the boats and ships that are parked in the harbour (Fernandez, 1983; Kennish, 1996; Cao et al., 2007). Copper showed wild array of essential role in haemoglobin biosynthesis and also it causes adverse effects of liver and kidney damage (Karunanidhi, 2017).

The concentration of cadmium fluctuated between 0.0148 and 0.14 ppm. Maximum cadmium concentration (0.14 ppm) was found in the site (T₁) while it was minimum (0.0148 ppm) at reference site (V). Palanichamy et al. (2004) recorded high concentration of cadmium in Arumuganeri waters which are very close to the study sites. The main source of cadmium to the marine environment is anthropogenic input and atmospheric loading (Kennish, 1996).

Fishes have the ability to accumulate Cd in various parts of the body tissues, especially in the kidney and liver, rather than muscle and skin. The amount of cadmium in tissues of various fish species is due to anthropogenic activities in the aquatic environment. The considerable variations in cadmium bioaccumulation may be due to the difference in their feeding habits. Higher concentration of in body organs causes harmful effects on reproduction, fertilization in aquatic animals (Karunanidhi, 2017).

Ajeeshkumar et al. (2013) reported that the sediment and water from southeast coast of India has elevated level of heavy metal when compared to permissible limit. In our present study cadmium which is measured to be more than permissible limits. Developmental activities along the coastal areas are the prime source of elevated level of heavy metal including large scale industries, textile mill and also attributed by municipal wastes, aquaculture and agricultural discharges (Karunanidhi, 2017).

In the study site, lead concentration in both the fish species was around 2.19 ppm and it was several order higher than the permissible limit recommended for human consumption by FAO and WHO (Table 4). A recent study revealed that the major accumulation of Pb was in kidney of the fishes (Karunanidhi et al, 2017). Lead is a non-essential element for living organism and also it causes various adverse effects such as neuro and nephro toxicity, rapid behavioural malfunction, and decrease in the growth, metabolism, and survival rate, alteration of social behavior in some mammals (Leston et al, 2010). Rashed (2001) found elevated Pb level in fishes obtained from fresh water ecosystem affected by extended agriculture, poultry

farms, textile, industrial and other activities. So, the sediments could be the major sources of Pb contamination and the bottom feeders may directly be affected with this deposited element in consequence to their feeding habit. The concentration of lead ranged from 1.9 to 2.19 ppm. Maximum concentration of lead (2.9 ppm) was recorded in the site (T_1), whereas it was minimum (1.9 ppm) at reference site (V). The higher concentration of lead observed in the present study may be due to the several sources such as boat exhaust systems, spillage of oil, and other petroleum products exhausted from mechanized boats employed in fishing and the discharge of sewage effluents from the industries of Thoothukudi into the water (Abu-Hilal, 1987 and Laxen, 1983).

The concentration of nickel in water samples was found to range from 2.4 to 2.96 ppm. Maximum concentration of nickel (2.96 ppm) was found in water samples of study site (T_1), whereas it was minimum (2.4 ppm) at reference site (V). The reasons mentioned for the high concentration of lead might be the reasons for the nickel concentration too. Overall, the metal analysis in the study area exhibited that the concentration of trace elements in the seawater in the study site are in the order of $Cu > Ni > Pb > Cd$.

5.5. Bioaccumulation of heavy metals

Bioaccumulation is defined as the uptake of toxicant by living cells and transport into the cell (Malik, 2004). Prosi (1979) revealed that metal enrichment in aquatic organisms was highly dependent upon the metal content in the water and a slight increase in the concentration in the water could lead to a significant increase in organism. Coastal waters tend to have higher concentration of heavy metals than

offshore waters. In most of the coasts, water movement is restricted and hence the concentration of pollutants builds up and the effects of pollution are more pronounced. The presence of xenobiotics in the marine environment exerts well-known biological effects on marine organisms. Fishes have been found to be good indicator of water contamination in aquatic systems because they occupy different trophic level; they are of different sizes and ages in comparison with invertebrates and also more sensitive to toxicant (Dallinger et al., 1987; Bark and Mason 1990; Burger et al., 2002).

As far as bioaccumulation of heavy metals is concerned, different tissues of fishes showed different capacities for accumulating heavy metals. The fishes collected from polluted sites showed higher levels of heavy metals in their tissues compared to same fish species collected from less polluted reference site. The highest metal concentration was found in the gill tissues of both fish species collected from both sites, followed by muscle. The bioaccumulation factor calculated for various heavy metals studied for *A. maculatus* in the study site was in the order of $Pb > Cd > Ni > Cu$ whereas it was $Cd > Cu > Ni > Pb$ for *T. puta*. From the study, it is learnt that ability of tissues to accumulate heavy metals in the tissues depends on the type of heavy metals, type of tissues and species (Mohamed et al., 2015).

The concentration of metals in the gill reflects the concentrations of metals in the waters where the fish species live. The absorption of metals at the gill surface, as the first target for pollutant in water, could also have an important influence on total metal levels within gills (Heath, 1987). The accumulation of lead, zinc and iron are high in the gills due to body's defense mechanism and this organ forms the

principal route for entry of pollutants from water. The metal concentration in muscle tissue is important for the edible parts of the fish. The mean concentrations of heavy metals analysed in the fish collected from less polluted sites was lower than the maximum permissible limits proposed by FAO (1983). There are several possible reasons explaining lower accumulation of metals in muscle. Firstly, the muscle does not come into direct contact with the toxicant medium as it is totally covered by skin which helps the organism avoiding the penetration of the toxicant. Similar results have been reported from a number of fish species that the muscle is not an active tissue in accumulating heavy metals (Mohammed et al, 2015).

Many authors reported that bioaccumulation factor for natural sea water is in the following order $Fe > Zn > Cu > Pb$. But for polluted waters, many reported that lead bioaccumulation was high followed by cadmium in aquatic organisms (Uma and Muthalagi, 2015; Karunanidhi et al., 2017). In the present study, the BAF was observed to be higher for cadmium than lead. BAF values were observed to be comparatively higher for *A. maculatus* than *T. puta* collected from sewage polluted study site, in both gill and muscle except cadmium in gill tissue of *T. puta*, BAF for cadmium in gill tissue of *T. puta* was 6.4 times higher than the water samples collected from study site (Fig 4.3.c and 4.3.d).

Anthropogenic activities like sewage runoff greatly influence the bioaccumulation of heavy metal in aquatic organism (Karunanidhi et al, 2017). The concentration of various trace metals estimated in the present study revealed its proximity with previous studies conducted along Tuticorin coast (Asha, 2010). In her study, the heavy metals were accumulated in the order $Fe > Mn > Zn > Cu > Pb > Cd > Ni$,

in the samples at three stations along Thoothukudi coast, which was in conformity with the observations of Ganesan et al. (1991) in Bay of Bengal. In her study the iron can was found to bioaccumulate 20 times more than the surrounding water, followed by copper three times and rest of trace metals.

5.6. Histopathology

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies (Hinton et al., 1992; Schwaiger et al., 1997; Teh et al., 1997). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer et al., 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta et al., 2003), and serve as warning signs of damage to animal health (Hinton and Lauren, 1990).

5.6.1. Histopathology of fish gill

Histology of gill tissue of both the fish species collected from reference site (Vembar) showed normal histoarchitecture (Plate 4.15.a and 4.16.a). The gill tissue of fish species collected from polluted site showed several histological alterations due to sewage pollution (Plate 4.17.a, 4.18.a, 4.19.a and 4.20.a).

The gills, are considered the primary target of the contaminants as they participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly

sensitive to changes in the quality of the water (Poleksic and Mitrovic-Tutundzic, 1994; Fernandes and Mazon, 2003). Marked variations like hyperplasia, vacuolation, deformation of cartilage core, bubbling of gill filament, epithelial lifting, lamellar fusion, secondary lamellar damage, shorter secondary lamellae and erosion of secondary lamellae were noticed in the gill tissues of *A. maculatus* collected from the polluted sites (Plate 4.17.a and 4.19.a).

The histological examination of gill of *T. puta* collected from the polluted sites exhibited the abnormalities like hyperplasia, mucous exordial, epithelial lifting and lamellar fusion, epithelial lifting, lamellar fusion, abnormalities like epithelial rupture, haemorrhage, vacuolation and deformation of cartilage core (Plate 4.18.a and 4.20.a).

Similar results were obtained by Winkaler et al. (2001) who noted several changes, such as lamellar aneurysm and lifting of the lamellar epithelium in gills of lambari (*Astyanax jacuhiensis*) from streams polluted by domestic sewage in Londrina, Paraná State, Brazil. Proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae and edema in secondary lamellae accompanied with separation of their epithelium from the lamellar supporting cells (Nowak et al., 1992). Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Mallatt, 1985;

Hinton and Laurén, 1990; Poleksic and Mitrovic-Tutundzic, 1994; Fernandes and Mazon, 2003). These alterations were most commonly found in the gills of fish caged in the polluted site from Corentyne coast. Coutinho and Gokhale (2000) found epithelial lifting in the gills of carps (*Cyprinus carpio*) and tilapias (*Oreochromis mossambicus*) exposed to the effluents of a wastewater treatment plant. Response of fish gill to stress/pollutant exposure seems not to be affected by the biology of the fish (sex and age) or seasonal factors (Gaber, 2013).

Liebal et al. (2013) investigated the histopathology of sewage polluted lakes located at Southern region of Brazil, and observed the histological alterations in gill like aneurysm, disruption of pillar cells, and hyperplasia on epithelial tissue, primary lamellar fusion, consequently, this will decrease the surface area for gas exchange. Rajeshkumar et al. (2015) also noted similar kind of histopathological damage in *Mugil cephalus* collected from heavy metal polluted Berbice-Corentyne coast of Guyana. Through the gills, as the main site of xenobiotic transfer, the toxins are distributed through their bodies accumulating in tissues and organs and may have deleterious effects (Vasanthi et al., 2013).

Mallatt (1985) reviewed that most common gill lesions found under several stressful conditions and reported the common changes like epithelial lifting, necrosis, hyperplasia, hypertrophy and hemorrhage as a of lethal conditions. A typical chronology of damage from acute exposure to the test chemical is first a lifting of outer layer of lamellar epithelium usually starting in the area of chloride cells. Edematous spaces are formed between the layers of epithelium and these may become infiltrated with leukocytes. Eventually, the whole epithelium sloughs off and

the lamellar loses rigidity on the blood side of the lamellar, the central space collapse, but the marginal channel often remains normal until the rest of the lamella is essentially destroyed (Heath, 1995). The proliferation of chloride cells are thought to be a compensatory response to iron loss. And therefore chloride cells hyperplasia may therefore be a good biomarker of adaptation. Hyperplasia of undifferentiated epithelial cells which results in clubbing and lamellar fusion is a much less specific lesion associated with a wide variety of unrelated insults (Hinton et al., 1998).

Santos et al. (2014) reported that the histology of fishes like *Centropomus undecimalis* and *Sardinella* sp collected from Jansen lagoon that receive domestic sewage water, the gill tissue of the above mentioned specimen showed histological alterations like lifting of the respiratory epithelium, hyperplasia of the lamellar epithelium, incomplete and complete fusion of several lamellae, disorganization of the lamellae, congestion of blood vessels, aneurysms, hypertrophy of the respiratory epithelium, hemorrhage and rupture of the lamellar epithelium and parasite. Afifi et al. (2014) studied the histological alterations in gill tissue of *Siganus canaliculatus* and *Epinephalus morio* caught from oil polluted Jeddah coast and observed congestion of blood vessel, focal hemorrhage, edema, epithelial swelling, and fusion of secondary lamellae, necrosis and aggregation of mucous cells.

Rajeshkumar et al. (2015) assessed the heavy metal pollution in east Barbic-Correntyne, Guyana by using histology of *Mugil cephalus* gill tissue the result and revealed alterations like aneurysm, mucous deposition, hypertrophy, fusion of secondary lamellae, ruptured epithelial layer, lifting of primary lamellae, lamellar swelling and necrosis.

5.6.2. Histopathology of fish liver

Histology of liver tissue of both the fish species collected from reference site (Vembar) displayed normal histoarchitecture (Plate 4.15.b and 4.16.b). The liver tissue of fish species collected from polluted site showed several abnormal changes due to sewage pollution (Plate 4.17.b, 4.18.b, 4.19.b and 4.20.b).

Fish liver is a very interesting model for the study of interactions between environmental factors and hepatic structures and functions. Liver of fish can be considered as target organ to pollutants, microorganisms etc, and alterations in its structure can be significant in the evaluation of fish health (Myers et al., 1988). Moreover, the liver plays a major role in complex enzymatic processes that are responsible for vital functions, such as accumulation and biotransformation of xenobiotics in the fish (Plate 4.15.b and 4.16.b).

Pathological changes in organs and tissues are generally characterized by an obvious increase in morphological and physiological, or functional, variability, both within a single organism and from one individual to another. This increased variability results from the more frequent occurrence of individual symptoms in a variety of combinations. Fish liver histopathology is an indicator of chemical toxicity and it is a useful way to study the effects of exposure of aquatic animals to toxins present in the aquatic environment (Fernandes et al., 2008). The severity of histological damages in any particular aquatic organism is directly proportional to the concentration of a pollutant in the medium. Moreover, the histopathological picture of the organs can corroborate with the biochemical changes accounting for the functional disruptions in the activity of the organs due to cellular damage.

The concentration of pollutants is more important in bringing the histological changes in the liver of fish; hence these changes could be used as a tool for assessing the toxic effects of the pollutants in aquatic environment (Jayantha Rao, et al., 1985). The extent of liver damage observed in the present investigation indicates that chronic exposure always causes impairment to the architecture of the tissue. Since liver is involved in detoxification of pollutants (Philip, *et al.*, 1989), it is susceptible to a greater degree of disruption in its structural organization due to toxic stress.

Some distinct changes like rupture of hepatocytes, melanomacrophages, increased Kupffer cell, increased pycnotic nucleus, vacuolation, ruptured nucleus, Blood congestion, cytoplasmic vacuolation and nucleus disorganization were observed in the liver of fish (Plate 4.17.b, 4.18.b, 4.19.b and 4.20.b).

Macrophage aggregates have been suggested as potentially sensitive histological biomarkers and or immunological biomarker of contaminant exposure (Schmitt and Dethloft, 2000). Histological changes observed in various studies in liver taken from exposed to pollutants include increased vacuoles in the cytoplasm, changes in nuclear shapes, focal area of necrosis (death of cells in a localized area), ischemia (blockage of capillary circulation), hepatocellular shrinkage, regression of hepatocytic microvilli at the bile canaliculi, fatty degeneration and loss of glycogen.

Bakhiet (2015) reported that fishes collected from sewage contaminated Nile river stretch showed histological changes in liver tissues due to bioaccumulation of heavy metal. The result revealed degeneration of the hepatocytes, congestion of central vein and nuclear pyknosis in the majority of hepatic cells. Similar results were

observed by Van Dyk (2003) Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment, or in other organs (Heath, 1995). These findings were apparent as the liver considered the organ of detoxification, excretion and binding proteins such as metallothionein. The metal-binding proteins present in the nuclei of hepatocytes suggested that the increase in the cell damages.

Coulibaly (2012) noted histological changes in liver of black finned tilapia from heavy metals contaminated Bay, Bietri like dilation in hepatic cells, focal areas of necrosis and cells hepatic degeneration, vacuolar degeneration with infiltration of lipid, blood vessels degeneration.

Agamy (2013) investigated the histological alterations in liver tissue of *Siganus canaliculatus* following the exposure to crude oil. The alterations included microvesicular lipid vacuolation, lipid accumulation, blood vessel congestion, nuclear and cellular degeneration, megalocytosis, karyomegally, bile stagnation, cholangitis, bile duct proliferation, lymphocytic infiltration, and spongiosis hepatis were noted. A study conducted by, in *Mastacembelus armatus* for the assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated water and the liver tissue showed alterations like unshaped hepatocytes, vacuolization, necrosis of parenchyma, pyknosis and infiltration of leucocytes(Javid and Uamani, 2013).

Afifi et al. (2014) studied the histological alterations in liver tissue of *Siganus canaliculatus* and *Epinephalus morio* caught from oil polluted Jeddah coast and reported that lipid aggregation, hydrophic degeneration, necrosis, pyknosis and karyorrhexis with cholangitis of proliferated bile duct epithelium, spongiosis hepatis,

Hepatocyte with enlarged nuclei with prominent nucleoli (megacytes). Congestion of hepatoportal blood vessels, intravascular hemolysis, hemorrhage and intravascular vacuolated leukocytes are seen in the liver tissue histology. A study conducted by Javid and Uamani 2013, in *Mastacembelus armatus* for the Assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated water the result Shows that severe lamellar fusion, hyperplasia, hypertrophy and epithelial lifting, swelling and deformed lamella, in some parts sloughing off and curving of lamellae was also observed.

Troncoso et al. (2012) studied the histological alterations in liver tissue of *Prochilodus lineatus* from the Salado River basin of Argentina, and the study revealed hemorrhage, vacuolar degeneration, nuclear alteration, fibrosis, necrosis, leukocyte infiltration, activation of macrophages. The liver tissues of *Mugill cephalus* collected from polluted Ennore estuary liver tissues showed histological alterations like vacuolization in the hepatocytes, fibroblast proliferation vacuole formation granular degeneration, and necrosis (Vasanthi et al., 2013).

Marchand (2012) reported that histopathological changes of fish liver from polluted freshwater system shows structural alterations in hepatic plates or cords, multiple focal areas of cellular alterations leading to a loss of uniform Hepatocyte structure, steatosis. Cytoplasmic and nuclear alterations (hypertrophic and pyknotic nuclei) of hepatocyte, increase in the size of melanomacrophage centers (MMCs), and focal areas of necrosis.

Feist (2015), investigated the histology of *Hoplostethus atlanticus* and *Coryphaenoides rupestris* collected from continental slope of northwest Atlantic

Ocean and the study revealed histological alterations of liver tissues like hepatocellular and nuclear pleomorphism and enlarged hepatocyte with loose granular cytoplasm, hypertrophied nuclei with coarse chromatin and granuloma. Rajeshkumar et al. (2015) assessed the heavy metal pollution in east Barbic-Correntyne, Guyana by using histology of *Mugil cephalus* liver tissue. The result showed the alterations like vacuolization in the hepatocytes, fibroblast proliferation, vacuole formation, granular degeneration, and necrosis.

5.6.3. Histopathology of fish Kidney

Histology of kidney tissue of both the fish species collected from reference site (Vembar) displayed normal histoarchitecture (Plate 4.15.c and 4.16.c). The kidney tissue of fish species collected from polluted site showed several abnormal changes due to sewage pollution (Plate 4.17.c, 4.18.c, 4.19.c and 4.20.c).

Teleostean kidney consists of head and body kidneys. Head kidney is the anterior portion of the kidney and consists of lymphoid tissue. Body kidney is composed of many nephrons and interstitial lymphoid tissue. The interstitial tissue is the major haematopoietic tissue in the body. Each nephron consists of two parts, the glomerulus (G) and the urinary tubule. The glomerulus capsule consists of an inner and outer layer of single flattened epithelia. Renal tubules (RT) consist of single layer of epithelial cells. Mesangium fills the space between the loops of glomerular capillaries (Plate 4.15.c and 4.16.c).

The posterior kidney of freshwater fishes is largely dedicated to the production of copious dilute urine and it has little responsibility for ion or acid-base balance. In marine fish, where they are opposite osmotic gradients, urine flow is

severely reduced by elimination of all but the proximal tubules. In some marine species, water loss is further reduced by elimination of glomerular filtration altogether and renal function depends solely on tubular secretion.

The kidney of the fish receives the vast majority of post branchial blood, and because of that, renal lesions can be expected in the fish when toxicant agents exist in the environment. Therefore, a study of these possible kidney changes may be expected to be a good indicator of environmental pollution.

In the present study, kidney of the fish showed swelling in tubule cells. This alteration can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm. Initial stage in the degeneration process can progress to hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Hinton & Lauren, 1990). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995). The presence of tubule degeneration, coupled with the absence of necrosis in the kidney in the present study indicates that the kidney suffered damage after exposure to pollutants. Some distinct changes like blood congestion, increased periglomerular space, increased peritubular space, glomerulus, degenerated tubules, shrunken glomerulus, necrosis, vacuolation, melonomacrophage, degenerated glomerulus, loss of cytoplasm were observed in the kidney of fish (Plate 4.17.c, 4.18.c, 4.19.c and 4.20.c).

Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration and changes in the corpuscle, such as

dilation of capillaries in the glomerulus and reduction of Bowman's space (Takashima and Hibiya, 1995). Following exposure of fish to toxic agents such as pesticides, histological alterations have been found at the level of the tubular epithelium and glomerulus (Teh et al., 1997). Similar alterations were found in fishes exposed to organic contaminants and mixed environmental contaminants (Schwaiger *et al.*, 1997; Pacheco & Santos, 2002)

Non-specific kidney histopathological lesions e.g. degenerative changes in tubular epithelium, dilation of tubular lumina, proteinaceous or cellular casts within tubular lumina, tubular necrosis and/or epithelial desquamation, and necrosis of interstitial hematopoietic tissues were observed following exposure of fish to organochlorines, petroleum compounds, organophosphate, herbicides and heavy metals (Garber, 2013). Kidney histopathology in some feral fish shows promise as an indicator of organic contaminant exposure (Myers et al, 1993).

Coulibaly (2012) noted the histological changes in kidney of black finned tilapia from heavy metals contaminated Bay Bietri and reported vacuolization, dilation in renal cells, severe dilation with renal cells degeneration, hemorrhage, parasitic cyst, vacuolar degeneration in the epithelium of renal tubules, parasites, dilation in renal blood vessels and cyst were observed. Gusma et al. (2012) reported that the larvae of Marine Pejerrey *Odontesthes argentinensis* exposed to water soluble fraction of petroleum resulted in histological alterations, like enlargement of glomerulus, intercellular spaces and abnormal nucleus of epithelial cells, and hypertrophy were noticed.

5.6.4. Histopathology of fish brain

Histology of brain of fishes collected from the reference site (Vembar) showed normal histoarchitecture (Plate 4.15.d, 4.16.d). The brain tissue of fishes collected from polluted site showed several abnormalities due to sewage pollution (Plate 4.17d, 4.18d, 4.19d and 4.20d).

The extent of damage to the brain was more in lethal than in sublethal concentrations exposed to pesticides, which may alters the physiological and behavioural functions of the fish. Various regions in fish brain are concerned with many functions such as feeding, vision, proper body motion can be directly related to the impaired neuronal dysfunction of central nervous system due to inhibition of brain AChE activity and all of them altered the complete behaviour and reduce the fitness of fish in its environments (Plate 4.15 and 4.16) .

In the present study histological investigation of brain tissues showed alterations like enlarged pyramidal cell, vacuolation, ruptured nucleus, degenerated nucleus, necrosis, and hemorrhage (Plate 4.17.d, 4.18.d, 4.19.d and 4.20.d) .

Das and Mukherjee (2000) reported that hexachlorocyclohexane was neurotoxic and induced vacuolation of brain parenchyma and moderate swelling of pyramidal cells of the cerebrum and opined that vacuolation may have been due to glycolysis leading to microsomal and mitochondrial dysfunctions. Losses of Nissl substances and glial cell reaction, with evidence of glial nodule formation in places, were proof of the neurotoxic nature of the chemical.

The vacuolation, dilation of blood capillary, fibrosis, agglutination of neurons and loss of definite demarcation between layers were observed on optic tectum of

Channa punctatus by Karuppasamy (2000). The above findings were also in support to the observation of Bhattacharya and Mukherjee (1978) in the optic tectum of *Clarias batrachus* and *Channa punctatus*.

Pugazhvendan et al. (2009) observed severe necrosis in brain tissue of *Ophiocephalus punctatus* exposed to Malathion pesticide. Patnaik (2010) observed histological alterations of brain tissue of common carp exposed to heavy metals like Pb, Cd. The changes observed were neuronal cell degeneration, dystrophic changes, swelling of pyramidal cells, loss of nasal substratum and vacuolation. Chamarthi et al. (2014) studied the histological alterations in brain tissue of *cyprinus carpio* exposed to quinalphos.

Histological changes in brain due to zinc toxicity in *Labeo rohita* exposed to 5 mg L⁻¹ showed swelling of pyramidal cells with binucleated nuclei and at 10 mg L⁻¹ exposure severe necrosis of neuronal cells of cerebrum was observed by Loganathan et al., (2006) indicating loss of nissl substances mild vacuolar changes with empty spaces appeared due to increased concentration and duration.

Histology of muscle tissue of both the fish species collected from reference site (Vembar) exhibited normal histoarchitecture (Plate 4.15e and 4.16e). The muscle tissue of fish species collected from polluted site showed several alterations due to sewage pollution (Plate 4.17.e, 4.18.e, 4.19.e and 4.20.e).

Muscle tissue composed of elongated muscle fibers, each an individual muscle cell, held together by connective tissues. Many individual muscles show an arrangement of fibers into bundles separated from each other by connective tissue portions. The nerves generally penetrate a muscle on its side and branch out as they

penetrate the connective tissue. Segmentation or metamerism of vertebrate musculature is seen clearly in the lateral muscles of the fishes. They are divided into myotomes or muscle segments, each of which is bent into a single V with the angle directed anteriorly. The function of these lateral muscles in almost all types of fishes is locomotion by swimming. Basically, alternate contractions passing posteriorly along the muscles on the two sides of the body result in the well known undulating movements of trunk and tail (Plate 4.15.e and 4.16.e).

In the present study histological investigation of muscle tissue showed alterations like splitting of muscle bundles, hyaline degeneration and degeneration of muscle bundles (Plate 4.17.e, 4.18.e, 4.19.e and 4.20.e). Muscles of fish collected from the polluted sites showed loss of muscle fibre as well as swelling of muscle fibre layer. Normal architecture of muscle bundle seems to be distorted with breakdown of muscle bundles. Earlier studies have demonstrated that muscles are very sensitive to contaminants and their immune system can act as an early warning system of stress (Anderson, 1990; Dyrzynda et al., 1997; Sauves et al., 2002).

Observations have been made by Nagarajan and Suresh (2005) in the muscle tissue of the fish *Cirrhinus mrigala* with increasing concentrations of sago effluent. Sakr and Gabr, 1991; Nour and Amer, 1995 and Das and Mukherjee, (2000) have studied the effect of different pollutants on fish muscles.

Simeon (2011) has observed mild lesion, necrosis, and inclusion of bodies, inflammation and cellular degeneration in the muscle tissue of the fish *Oreochromis niloticus* exposed to aqueous and ethanolic extracts of *Ipomoea aquatica* leaf. Patnaik et al. (2011) studied the histopathology of fish *Cyprinus carpio*

exposed to sublethal concentrations of lead and cadmium. The fish showed marked thickening and separation of muscle bundles with severe intracellular edema. Similar observation has been made by Das and Mukharjie (2000).

Ibrahim et al. (2013) studied Bioaccumulation of heavy metals and their histopathological impact on muscles of *Clarias gariepinus* from El-Rahawy drain, Egypt the result shows that, hyaline degeneration, edema, and splitting muscle bundles. Afifi et al. (2014) studied the histological alterations in muscle tissue of *Siganus canaliculatus* and *Epinephalus morio* caught from oil polluted Jeddah coast and observed slight edema, hyalinization and necrosis.

Rajeshkumar et al. (2015), studied to assessed the heavy metal pollution in east Barbic-Correntyne, Guyana by using histology of *Mugill cephalus* muscle tissue. The study revealed alterations like loss and swelling of muscle fibre layer, breakdown of muscle bundles, splitting of muscle fibres. The muscle tissues of *Mugill cephalus* collected from the polluted Ennore estuary showed histological alterations like necrosis in the muscle bundles, connective tissue damage, splitting of muscle fibres and edema between muscle bundles (Vasanthi et al., 2013).

VI. SUMMARY AND CONCLUSION

Thoothukudi is an industrial town which is situated in the Gulf of Mannar, Bay of Bengal, Southeast coast of India. It was famous for pearl fishing three decades back. This region has the richest littoral fauna. There are a number of coral islands in this zone. This marine park is facing a lot of threat to its fertility. The pearl fishing has come to an extinct. The effluents from the major and minor industries situated in and around Thoothukudi let out the treated effluents into the sea. Also the untreated sewage is let into the coastal waters. These bring in excess nutrients and high levels of heavy metals into the seas in the form of untreated or partially treated effluents. Though there are some stray reports available for the Thoothukudi coast, there is no comprehensive information on the fish faunal composition, status of physico-chemical properties of seawater, nutrients, trace metals and their influence on the histology of various organs of fishes. Hence, the present study was undertaken to understand the impacts of the sewage pollution that takes place in the sewage disposal site, Therespuram off Thoothukudi coast on the histology of various organs of finfish species viz., *Arius maculatus* and *Terapon puta*. Vembar, situated north of Thoothukudi district was selected as relatively unpolluted reference site. The outcomes of the research work are as follows:

- A total of 400 specimens that included 30 species of finfishes, 8 species of shellfishes and one species of sea urchin (Plate 4.1) were recorded during study period. The finfishes recorded were found to belong to seven orders with the dominance of the order Perciformes (9 families, 17 species), followed by Siluriformes (1 family, 6 species), Clupeiformes (2 families, 3 species). Each of the remaining orders like Pleuronectiformes, Tetradontiformes, Elopiformes and Mugiliformes were represented by one species. The shellfishes recorded included two species of shrimp (Family: Penaeidae), two species of crab (Family: Portunidae) and four species of gastropod (Family: Muricidae). In

addition, only one sea urchin, species belonging to the family, Temnopleuridae under the order Camarodonta was recorded.

- Seawater samples were collected bimonthly from five different spots designated as T₁, T₂, T₃, T₄ and T₅ in the sewage disposal site, Therespuram off Thoothukudi coast and also from the reference site, Vembar (V) from Sep; 2016 to June' 2017 for the analysis of various physico-chemical properties of seawater and for the generation of GIS based sewage spread rate map.
- ANOVA (one way) of data gathered on the various physico-chemical properties of seawater such as temperature, salinity, pH, alkalinity, BOD, COD, TDS, TSS, ammonia, nitrate, nitrite, phosphate and silicate showed significant difference between any two study sites (among T₁, T₂, T₃, T₄ and T₅) and also between anyone of the study site and reference site at 0.05% level for each parameter. Besides, they were noted to be positively correlated and the regression analysis showed that there is a significant difference between the study site and reference site at 0.05% level for each parameter. Spatio-temporal variations could be observed for all the physico-chemical parameters in the study site. Further, it should be noted that all these parameters at the study site were not within the permissible limit of the International standards except temperature. However, they were found to be within the limits in the reference site, Vembar (V).
- GIS based spatial variability map of different seawater quality parameters was generated for the study site for summer and monsoon seasons separately using interpolation operation in the Arc-GIS software. From the sewage spread rate map, it is learnt that there is a gradual reduction in nutrients towards the sea.
- Heavy metals such as copper, cadmium, lead and nickel were estimated in water samples collected from exact point of sewage disposal at Therespuram (T₁), off Thoothukudi coast and reference site (Vembar) during summer season. Statistical analysis of (One way ANOVA) of the concentration of all the heavy

metals in water samples of study site T₁ and reference site (V) showed significant difference between the study site and reference site at 0.05% level.

- It should be noted that the values of heavy metals such as copper, cadmium, lead and nickel recorded from the study site during the study period were not within the permissible limit of the international standards. However, the concentration of same heavy metals were within the permissible limit of International standards in reference site (Vembar) except lead which might be due to berthing of fishing boats along the shore.
- As far as bioaccumulation of heavy metals is concerned, different tissues of fishes showed different capacities for accumulating heavy metals. The fishes collected from polluted sites showed higher levels of heavy metals in their tissues compared to same fish species collected from less polluted reference site. The highest metal concentration was found in the gill tissues of both fish species collected from both sites, followed by muscle. The bioaccumulation factor calculated for various heavy metals studied for *A. maculatus* in the study site was in the order of Pb > Cd > Ni > Cu whereas it was Cd > Cu > Ni > Pb for *T. puta*. From the study, it is learnt that ability of tissues to accumulate heavy metals in the tissues depends on the type of heavy metals, type of tissues and species.
- Histology of gill, liver, kidney, brain and muscle tissues of *A. maculatus* and *T. puta* collected from reference site (Vembar) showed normal histoarchitecture, whereas several histopathological alterations were observed in all these tissues from the same fish species collected from the study site.
- Histological sections of organs such as gill, liver, kidney, brain, and muscle tissues were prepared from of *A. maculatus* and *T. puta* collected from sewage disposal site bimonthly during Sep 2016 to June 2017. The observations of the histological sections observed under light microscope revealed several tissue specific alterations in common in all the organs studied. However, the intensity of

the alterations was observed to be the highest in the fish specimens collected during the summer season and the lowest in fish specimen collected during monsoon season. Further, most of the histological changes were observed to be similar for both fish species.

- Histology of gill, liver, kidney, brain and muscle tissues of *A. maculatus* and *T. puta* collected from reference site (Vembar) revealed normal histoarchitecture, whereas several histopathological alterations were observed in all these tissues from the same fish species collected from the study site.
- Marked abnormal variations like hyperplasia, vacuolation, deformation of cartilage core, bubbling of gill filament, epithelial lifting and lamellar fusion, secondary lamellar damage, shorter secondary lamellae and erosion of secondary lamellae were noticed in the gill tissues of the fishes.
- Abnormalities like blood congestion, cytoplasmic vacuolation, rupture of hepatocytes, vacuolation, degenerated nucleus and nucleus disorganization were observed in the liver tissues of fishes.
- The histopathological analysis of kidney of fishes showed blood congestion, cytoplasmic vacuolation, vacuolation, increased periglomerular space, degenerated glomerulus and increased peritubular space and melanomacrophage.
- Histopathology of brain of fishes exhibited enlarged pyramidal cell, vacuolation, binucleated nucleus, ruptured nucleus, and haemorrhage.
- The histopathological investigation of muscle tissues of fishes showed some remarkable changes like splitting muscle bundles, hyaline degeneration, degeneration of muscle bundles and vacuolar degeneration.

It is evident from the present study that histopathological markers can be proposed as an efficient and sensitive method to monitor fish health and environmental pollution in natural water bodies.

The coastal areas of Thoothukudi assume greater importance due to increasing human population, urbanization and accelerated industrial activities. These anthropogenic activities have put tremendous pressure on the fragile coastal ecosystems. The coastal pollution has highlighted the need to deploy appropriate biological indices and measurements for these locations. In this context, the present study has proved that histopathological alterations take place in common fish species captured from sewage polluted coastal regions. Many of these changes represent a biological endpoint of contaminant exposure and as such, several studies reinforce the application of histopathology as a powerful tool for monitoring anthropogenic contamination in the coastal regions. Whilst links between such histopathological alterations and contaminants are not definitive, such surveillance provides a useful insight into individual, population and overall ecosystem quality. The present research work has reported biological endpoint data for multi-organ histopathological alterations in selected species of marine fish species such as *Arius maculatus* and *Terapon puta* captured from the sewage disposal site in Therespuram off Thoothukudi coast and make recommendations for the application of multi-organ histopathology to future biological effects monitoring programmes of this type. Furthermore, in the future, when these histopathological endpoints are assessed in conjunction with other parameters such as parasite community structure, enzyme responses, bile metabolite levels and molecular damage indices, a clearer picture of the complex interactions between anthropogenic and natural environmental modifiers would definitely emerge.

VII. REFERENCES

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